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SYNONYMY IN SOME COMMON SPECIES OF CERCOSPORA[1]

E. M. JOHNSON AND W. D. VALLEAU

(Accepted for publication May 1, 1949)

Frogeye of tobacco, usually attributed to the fungus *Cercospora nicotianae* Ell. and Ev., is a common but seldom destructive disease, usually on lower leaves of plants in humid locations. In a damp autumn, infections may occur on higher leaves and, if the crop is cut before it is fully ripe, green spots (2) may appear on the cured leaves; but if the crop is allowed to ripen, the upper leaves may suddenly develop large necrotic spots and be quickly destroyed unless cut immediately. Frogeye tobacco is usually not discounted on the market, because buyers take the view that the spots are an indication of a ripe crop.

Careful observations at setting time over a period of 25 years have shown that frogeye is an extremely rare disease in plant beds in Kentucky. We occasionally have found a single frogeye spot at the time of first pulling, but usually all plants in the beds appear to be completely free from infection. Yet in the field the disease is comparatively common late in the season.

These observations raise the question whether the causal agent, *Cercospora nicotianae,* is strictly a pathogen of tobacco or whether it might not also be a pathogen of other plants from which wind-borne spores might be carried to tobacco. Whether *C. nicotianae* is a valid species, restricted in its host range, is of fundamental importance to the pathologist who is trying to determine the source of infection. The same, of course, is true of the fungi causing leaf spots of beet, celery, and other commercial crops that are regularly injured by cercospora leaf spot. If *C. nicotianae* attacks only tobacco and closely allied plants, then control measures can be based largely upon a study of the organism in relation to tobacco. If, however, *Cercospora* from numerous host species attacks tobacco, as Vestal (8) has shown for sugar beets, then the disease on tobacco must be studied from the standpoint not only of tobacco but of the native vegetation as well.

The purpose of this report is to show that the inoculum for outbreaks of frogeye in tobacco need not arise from a previous tobacco crop, but may originate on several unrelated plants growing in the vicinity of tobacco plant beds and fields; and that, as a consequence, the usual concept of species in *Cercospora* must be revised.

MATERIALS AND METHODS

Cultures of *Cercospora* were obtained by streaking on potato-dextrose agar conidia from cercospora leaf spots from 28 species of plants in 16

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

TABLE 1.—*Results of inoculations with Cercospora isolated from crop plants and weeds*

Source of isolate	Number of plants infected : number inoculated[a]							
	Tobacco	Bean	Cabbage	Beet	Petunia	Nicotiana rustica	Pansy	Cantaloupe
Solanaceae								
Nicotiana tabacum L.	17/19	6/11	11/15	15/23	3/11	43/52	.....	0/1
Petunia hybrida Vilm.	1/3	3/4	2/3	4/6	0/4	7/12	.....	.....
Physalis subglabrata Mack. and Bush.	2/3	2/3	1/1	1/3	1/2	0/3	.....	1/1
Campanulaceae								
Campanula sp.	1/1	0/5	1/2	.....	.....	1/1	1/1	0/1
Chenopodiaceae								
Beta vulgaris L.	0/1	5/6	3/3	4/4	1/3	1/3	.....	0/1
Chenopodium album L.	.....	3/3	0/1	1/1	0/2	.....	.....	.....
Compositae								
Chrysanthemum parthenium Pers.	.....	1/1	0/1	1/1	0/1	0/2	.....	.....
Coreopsis sp.	1/1	0/3	0/1	.....	0/1	1/2	1/1	0/1
Convolvulaceae								
Ipomoea hederacea Jacq.	1/1	1/1	2/2	.....	.....	0/1	.....	0/1
Cruciferae								
Brassica oleracea var. *capitata* L.	2/2	2/2	2/4	1/1	1/2	1/1	.....	.....
Barbarea vulgaris R. Br.	1/1	3/3	0/2	.....	.....	.....	.....	.....
Cucurbitaceae								
Cucurbita pepo L.	1/3	1/2	2/2	4/4	2/3	10/11	.....	0/1
Cucumis sativus L.	.....	1/4	1/2	.....	.....	1/1	.....	1/1
Euphorbiaceae								
Ricinus communis L.	.....	1/1	0/1	.....	0/1	.....	.....	0/1
Gramineae								
Festuca elatior var. *arundinacea* (Schreb.) Wimm.	.....	1/1	1/1	1/1	0/1	.....	.....	0/1

TABLE 1—(*Continued*)

Source of isolate	Number of plants infected: number inoculated[a]							
	Tobacco	Bean	Cabbage	Beet	Petunia	Nicotiana rustica	Pansy	Cantaloupe
Labiatae								
Lamium amplexicaule L.	1/1	3/3	2/2	2/2	1/1	4/4	2/2	0/1
Leguminosae								
Lathyrus latifolius L.	1/1	2/3	2/3	2/2	1/1	0/2	1/1	.....
Lupinus polyphyllus Windl.	1/2	4/4	2/3	6/7	3/4	11/16	1/1	1/1
Phaseolus vulgaris L.	1/2	4/6	3/4	.....	1/2	0/1	1/1	1/1
Trifolium hybridum L.	0/1	0/4	0/2	1/1	0/1	.....	.....	0/1
Trifolium repens L.	.....	1/2	.....	.....	.....	.....	.....	.....
Malvaceae								
Malva rotundifolia L.	0/1	1/3	1/2	0/1	0/2	1/3	.....	0/1
Althaea rosea Cav.	0/1	0/1	.....	.....	.....	0/1	.....	.....
Phytolaccaceae								
Phytolacca decandra L.	2/3	1/5	2/2	1/4	0/3	2/8	.....	1/1
Plantaginaceae								
Plantago lanceolata L.	0/2	2/5	0/1	1/1	0/1	4/8	.....	0/1
Polemoniaceae								
Phlox paniculata L.	1/2	.....	2/2	.....	.....	1/1	.....	.....
Rosaceae								
Potentilla sp.	0/1	.....	0/2	1/1	1/1	0/2	.....	1/1
Rosa sp., var. Blaze	.....	0/1	2/2	0/1	.....	1/1	2/2	.....
Rubus allegheniensis Porter	0/1	1/1	0/2	.....	.....	1/2	.....	0/1
Violaceae								
Viola sp.	1/3	3/4	5/6	3/3	1/2	6/7	.....	.....

[a] Numerator—number of plants infected; denominator—number of plants inoculated.

families (Table 1). The isolates were grown on tobacco agar (3) at 27° C. in the dark. Inoculations were made to tobacco, *Nicotiana rustica*, and to other plants (Table 1). Mycelial cultures were used as inoculum, as spores were sparse or absent. Actively growing potted plants were inoculated by dipping their leaves in a mixture of 10 cc. of liquid soap in 400 cc. of water as a wetting agent, then in a suspension of a 3- to 5-day-old tobacco agar culture prepared by squeezing the agar mat through cheesecloth. After 48 hr. in a moist chamber the plants were kept in the greenhouse until spots developed, when they were returned to the moist chamber for 24 hr. Cercospora conidia produced on the spots were considered proof of pathogenicity. Camera lucida drawings were made of spores from culture and from inoculated plants for ready comparison of the so-called species.

RESULTS

In culture, colonies of the isolates from the various hosts were so similar that they could not be distinguished. Variations in color and in the amount of aerial mycelium between the so-called species were matched by variation within the isolates from a single host. Camera lucida drawings of spores and sporophores from the various hosts on which the isolates were grown gave no basis for separation of any of the isolates. From the standpoint of colony characters and morphology of conidia and conidiophores produced in culture, all of the isolates appear to be of a single species.

Frogeye spots that developed on Burley tobacco from inoculation with isolates from 16 host species in 11 families (Table 1) appeared alike and were typical of the disease as it occurs in the field. Measurements of spores and conidiophores from the lesions varied as much among the isolates from a species as among isolates from different species. Some differences were indicated in degree of pathogenicity among the isolates tested, but these differences were no greater than those among the 6 isolates from tobacco.

Scotia bean became infected when inoculated with isolates from 23 plant species in 15 families. The spots were alike and there were no consistent differences in spore measurements.

Isolates from 19 plant species in 14 families produced infection on Wisconsin All Seasons cabbage. The spots were grayish, depressed, irregular to circular, and varied from 4 to 10 mm. in diameter. Often an entire leaf was killed. Neither conidia nor conidiophores from the different isolates could be distinguished.

Seventeen of 19 isolates from 12 families caused typical cercospora leaf spot on Detroit Red beet. The spots from different isolates could not be distinguished, and again spores and conidiophores could not be distinguished.

Isolates from 11 species representing 8 families caused infection on

Heavenly Blue petunia. Two isolates from petunia failed to cause infection on petunia but they infected tobacco, bean, cabbage, and *Nicotiana rustica*.

Seventeen isolates from different host species representing 15 families caused typical frogeye infection on *Nicotiana rustica*. The spots were somewhat smaller than those occurring naturally in the field. Again the isolates could not be distinguished on the basis of conidiophores or conidia.

Only 7 isolates from different plants in 5 families were tested on Alpine Glow pansy, but all 7 caused spots typical of those that occur on *Viola sp.* in nature. Isolates from 6 plant species of 19 tested caused infection on cantaloupe.

It is evident from these inoculation tests that several so-called species of *Cercospora* from unrelated hosts cause typical frogeye on tobacco.

SYNONYMY IN CERCOSPORA

Mycologists seem to have assumed a high degree of specificity of *Cercospora* and a relatively high degree of stability of the organism in size of spores and conidiophores. When *Cercospora* is found on a plant on which it has not previously been reported, this is taken as sufficient basis for announcing a new species. If *Cercospora* has already been described on this host, but the collection does not correspond in morphological characters with the one described, this seems to justify the description of a new species, regardless of the fact that there may be morphologically similar species on members of many other plant families or even on other genera and species within the family.

While parasitism seems to be considered of first importance in mycological literature, *Cercospora* is in fact a weak parasite living, as Atkinson (1) pointed out, on dead, dying, languid, physiologically diseased tissues with perhaps occasional injury to healthy plants. Recognizing that different hosts, because of structural differences, might exert a powerful influence upon the form and characteristics of *Cercospora*, and considering that these might be effective agencies in producing variations in the parasite, Atkinson conceived that during a long period of time a few forms widely distributed over a great number of hosts may become more and more unlike and more firmly fixed in the possession of peculiar characteristics. He was assuming, without evidence, that the various forms remain generation after generation on the same host; whereas the results of cross-inoculation studies make it almost certain that any one of the forms of this semiparasitic organism are continually moving about from one host species to another. Thus changes in morphology are merely temporary ones forced upon the fungus by the temporary host. A fungus living under these conditions could hardly possess a high degree of host specificity.

That neither host nor minor differences in morphology are sound bases

for classifying *Cercospora* is suggested by the work of Welles (9). His inoculation of 13 species representing 12 genera of plants in 5 families with *Cercospora* from *Phaseolus lunatus* resulted in infection on all of the species. Welles found that spore size on the different genera varied enormously and concluded that morphological differences, unless exceedingly pronounced, are of little value as taxonomic criteria. He further concluded that the only valuable taxonomic criteria which have presented themselves for use in separating various species of *Cercospora* are physiological behavior on artificial media and extent of parasitism.

Vestal (8) found that 26 species of plants distributed throughout 12 widely separated families could be infected in the greenhouse by spraying with suspensions of conidia of *Cercospora beticola*, clearly indicating a wide host range of the organisms among plants of the sugar beet area. In the field, Vestal found six species of plants in six widely separated families infected with *Cercospora* morphologically and pathogenically similar to *C. beticola*. There is a possibility that 24 so-called species of *Cercospora* may be involved in the beet disease alone. Hill's (4) extended study of cercospora leaf spot of tobacco in Australia, in both greenhouse experiments and observations out-of-doors, convinced him that "only under exceptional circumstances do members of the Solanaceae other than the Nicotianae act as alternative hosts for the species of *Cercospora* affecting tobacco. In the majority of seasons they are not important."

The experimental results reported in this paper and the results of Welles (9, 10), Vestal (8), and Horsfall (5) indicate that some species of *Cercospora* have an extremely wide host range and that many so-called species that have been considered distinct are in fact one and the same. According to Lieneman (6), the type species of the genus *Cercospora* is *C. apii* Fres. Vestal (8) showed that *Apium graveolens* readily developed typical cercospora leaf spot when inoculated with *C. beticola*. That the two fungi are identical morphologically is well illustrated by Schwarze (7) in his drawings of conidia from the two species. It should be mentioned that while Schwarze described the spores of *C. apii* as clavate, on the basis of notes of W. S. Krout, and those of *C. beticola* as obclavate, the term clavate is obviously an error since Saccardo and others describe *C. apii* spores as obclavate. In our work we have been able to find no real morphologic differences between *C. beticola* and *C. nicotianae;* and in the inoculation studies of Vestal and in the present study both species caused infection on nine families in common, indicating that both have a very wide and similar host range. There is, therefore, ample evidence for considering *C. apii, C. beticola,* and *C. nicotianae* identical. In the limited cross-inoculation work that has been carried on, a relatively small number of so-called species of *Cercospora* has been studied. Yet the studies have been sufficient to show that the forms worked with are omnivorous and undoubtedly attack many more species of plants than we have had an oppor-

tunity to study. We propose, therefore, that all species of *Cercospora* having the general morphologic characteristics of *C. apii* be cast in synonymy with this species until proof is forthcoming that they are different. This probably will include several hundred names.

The species listed below are morphologically similar to *Cercospora apii* and on their respective hosts either *C. beticola* or *C. nicotianae* cause typical cercospora spots. They are considered synonymous with *C. apii: C. acetosellae* Ell., *C. alabamensis* Atk., *C. althaeina* Sacc., *C. anthelmintica* Atk., *C. arcti-ambrosiae* Halst., *C. avicularis* Wint., *C. bliti* Tharp, *C. bloxami* Berk. and Br., *C. brachiata* Ell. and Ev., *C. canescens* Ell. and Mart., *C. chrysanthemi* Heald and Wolf, *C. citrullina* Cooke, *C. columnaris* Ell. and Ev., *C. convolvuli* Tracy and Earle, *C. cruenta* Sacc., *C. cucurbitae* Ell. and Ev., *C. diffusa* Ell. and Ev., *C. festucae* Hardison, *C. filispora* Peck, *C. flagellaris* Ell. and Mart., *C. ipomoeae* Wint., *C. kellermani* Bubák, *C. lathyri* Dearn and House, *C. lathyrina* Ell. and Ev., *C. longispora* Peck, *C. lupini* Cooke, *C. lupinicola* Lieneman, *C. omphakodes* Ell. and Holw., *C. phaseolorum* Cooke, *C. phyllitidis* Hume, *C. physalicola* Ell. and Barth., *C. physalidis* Ell., *C. plantaginella* Tehon, *C. plantaginis* Sacc., *C. polygonacea* Ell. and Ev., *C. ricinella* Sacc. and Berl., *C. rosigena* Tharp, *C. rubi* Sacc., *C. septorioides* Ell. and Ev., *C. solanicola* Atk., *C. violae* Sacc., *C. viridula* Ell. and Ev., *C. zebrina* Pass.

SUMMARY

Frogeye of tobacco, caused by a species of *Cercospora*, though a rare disease in plant beds in Kentucky at pulling time, is a common and sometimes severe disease in the field. This raised the question as to whether inoculum in field outbreaks came from a previous tobacco crop or from other plants on which the fungus might also be parasitic.

Cultures of *Cercospora* were obtained from 28 species in 16 plant families. The isolates from the various hosts could not be distinguished by colony characters or by spore size or shape. Typical frogeye developed on tobacco inoculated with isolates from 16 species in 11 families. Similar results were obtained on other species of plants.

Neither pathogenicity based on host range nor morphology has been given sufficient consideration in naming new species of *Cercospora*. Species are usually named for the plant on which they are found. The evidence obtained in this study indicates that the species of *Cercospora* that attacks tobacco is omnivorous, occurring on many other host species. As *C. apii* Fres. appears to be the type species of the genus, and as *C. nicotianae* and *C. beticola* appear to be identical morphologically and pathogenically with it, it is proposed that these species and other species morphologically similar to *C. apii* be cast in synonymy with it until evidence to the contrary is forthcoming.

KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KENTUCKY

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EVALUATION OF RESISTANCE AND SUSCEPTIBILITY IN GARDEN PEA TO NEAR-WILT IN THE GREENHOUSE[1]

D. G. WELLS, W. W. HARE,[2] AND J. C. WALKER[3]

(Accepted for publication May 25, 1949)

The common garden pea (*Pisum sativum* L.) is subject to two vascular fusarial diseases, wilt and near-wilt, incited by *Fusarium oxysporum* f. *pisi* (Linford) race 1 Snyder and Hansen, and *F. oxysporum* f. *pisi* (Linford) race 2 Snyder and Hansen, respectively. Wilt was once a serious disease but adequate resistance was reported by Wade (5) in 1929 and was later incorporated into commercially important field and canning pea stocks. According to Walker and Hare (6), near-wilt, described in detail by Snyder and Walker (3) in 1935, became a disease of major importance in Wisconsin by 1942. Finding of resistance to near-wilt was reported by Hare *et al.* (1) in 1949 and is being currently used in the production of new varieties at the Wisconsin Agricultural Experiment Station.

During the early period of breeding for resistance to near-wilt, tests for disease reaction were conducted on soil naturally infested with the organism. This system was unsatisfactory because environmental conditions were often unfavorable for the complete elimination of near-wilt susceptible plants (1). A more dependable test for reaction to near-wilt was needed in order that the breeding program might be properly conducted.

Virgin and Walker (4) demonstrated in 1939 that Wisconsin Perfection peas grown in the greenhouse in soil either naturally or artificially infested with the near-wilt organism developed severe symptoms of near-wilt in 52 days from time of planting. Soil temperatures ranging from 20° to 32° C. were maintained. By the time adequate notes on disease development could be secured, the plants had grown so tall as to be difficult to handle and greenhouse space was not being efficiently utilized. This method of testing in the greenhouse for reaction to near-wilt was thus unsatisfactory for use in a breeding program.

The purpose of this paper is to report experiments made to establish a rapid test for reaction to near-wilt under controlled conditions in the greenhouse.

METHODS AND MATERIALS

Two varieties were used in these experiments, Delwiche Commando, a recent release of the Wisconsin Agricultural Experiment Station, resistant to wilt and near-wilt, and Wisconsin Perfection, a standard wilt resistant

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The illustrations were prepared by Eugene Herrling.

[2] Formerly Research Assistant and Assistant Professor, respectively, Departments of Agronomy and Plant Pathology, University of Wisconsin.

[3] Professor of Plant Pathology, University of Wisconsin.

canning variety, susceptible to near-wilt. These varieties have been shown by Hare *et al.* (1) to differ by a single factor for reaction to near-wilt.

Inoculum was obtained by growing the fungus in 250-ml. Erlenmeyer flasks containing 100 ml. of modified Czapek's solution.[4] Flasks containing inoculum were placed on a mechanical shaker for two days and then the contents of one flask, observed to be free of contaminants, were used to inoculate 3 liters of sterile Czapek's solution contained in a 6-liter flask. Two or three such quantities of nutrient solution were inoculated in this manner and placed on a mechanical shaker for 3 days. After this period of agitation the contents of the flasks consisted of a heavy suspension of spores and mycelial fragments.

Plants were grown in two types of containers. Metal pans, $3 \times 12 \times 18$ in., were filled with clean white quartz sand to within about ½ in. of the top. Glass siphons of the type described by Schroeder and Walker (2), installed at one end of each pan, were used in the first experiments performed but were found to be unnecessary if care was taken not to apply an excess of liquid. The pans were inserted into Wisconsin soil-temperature-control tanks.

The sand was flooded with distilled water in preparation for planting and the excess was siphoned off. A planting board was used that punched 12 holes per row, one row at a time, just deep enough for the seed to be easily covered, a distance of about ¼ in. Eight rows were planted in each pan. Shallow planting was found to be desirable for uniform emergence even though some of the seeds were forced out of the sand by the growth of the primary root before it was anchored by root hairs. Such seeds had to be placed back in the sand. Whenever the pans needed moisture they were sprinkled with nutrient solution.

Seven to 10 days from time of planting the seedlings were removed from the pans and treated as follows. Seedlings from one row (12 seedlings) of each of the varieties were immersed in distilled water, the tap roots clipped to a uniform length of 1 in., and the seedlings replanted in the pan. The roots of seedlings from two other rows were immersed in a heavy suspension of spores and mycelial fragments of the near-wilt fungus and then returned to the pan. Seedlings from the remaining rows were similarly treated except that the tap roots were clipped to a length of 1 in. while immersed in the inoculum and were then transplanted in the sand. Thus two of the eight rows were for control purposes, two were inoculated by dipping, and four were inoculated by clipping.

The clipping method of inoculation was later improved. The roots were washed in distilled or tap water and the seedlings laid a row at a time on a cutting board. This board had a groove running lengthwise deep enough to accommodate the cotyledons and to make the seedlings lie at a uniform distance from another, but narrower, groove over which the roots extended. The board was then laid on the surface of inoculum in a shallow baking

[4] KNO_3, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4.7H_2O$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, trace; cerelose, 30 gm.; water, 1,000 ml.

pan and a slender stick was placed over the seedlings to prevent their floating when the board was immersed. After immersion, the tap roots were severed by inserting one end of a razor blade in the narrow groove and

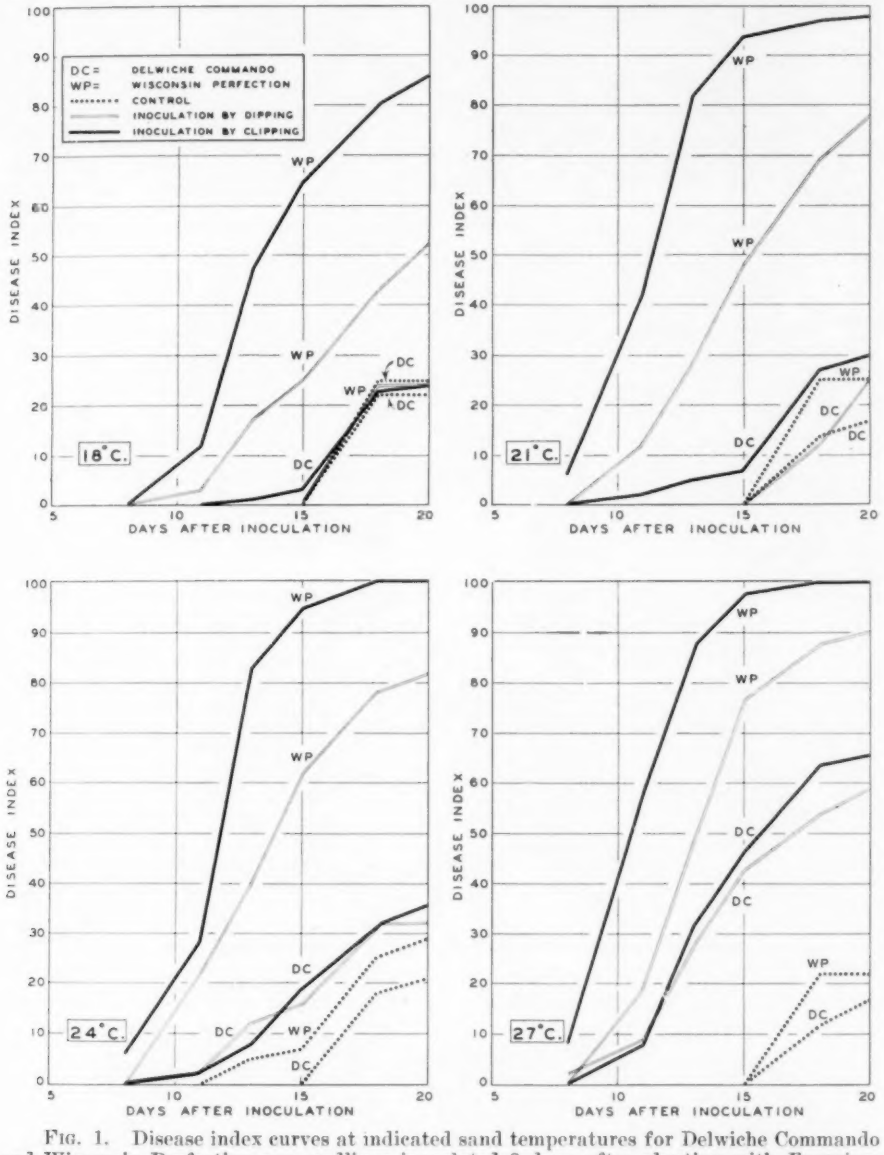

FIG. 1. Disease index curves at indicated sand temperatures for Delwiche Commando and Wisconsin Perfection pea seedlings inoculated 8 days after planting with *Fusarium oxysporum* f. *pisi* race 2.

pulling it toward the operator so as to cut all of the tap roots about 7/8 in. from the cotyledons. A row of 12 holes 1½ in. deep were then punched in the sand and the inoculated seedlings were quickly and easily transplanted. When the transplanting was completed the pan was returned to the tem-

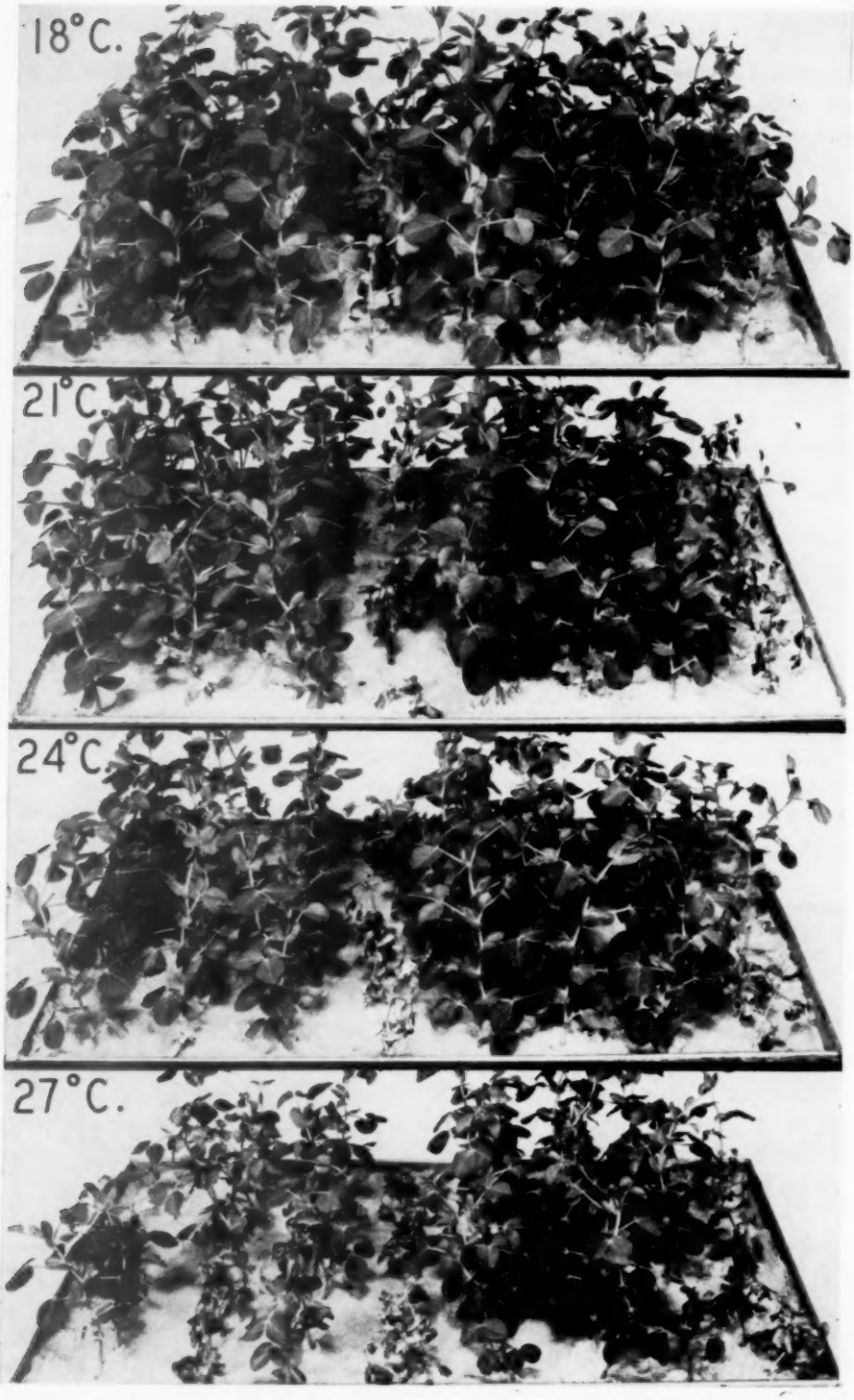

FIG. 2. Comparative development of near-wilt of peas at the indicated temperatures 20 days after inoculation with *Fusarium oxysporum* f. *pisi* race 2. Rows (numbered from left to right) 1, 3, 5 and 7, Delwiche Commando; rows 2, 4, 6, and 8, Wisconsin Perfection. Rows 1 and 2 inoculated by the dip method; rows 3 and 4, 7 and 8, by the clip method. Rows 5 and 6 were not inoculated.

perature tank and lightly sprinkled with nutrient solution, care being taken not to overwater. If the sand was wet enough so that water could be siphoned off, then a siphon was set in place and as much water was removed as possible.

Tank temperatures were adjusted to maintain sand temperatures of 18°, 21°, 24°, and 27° C., while a common air temperature of about 22° C. was maintained. The seedlings were examined at 8, 11, 13, 15, 18, and 20 days after inoculation and each was assigned to one of four disease classes: Class 1, healthy—normal color and development; Class 2, slightly diseased—one stipule of a pair smaller than the other, or yellowing and dying of one-half or fewer of the leaves at the plant base and upward; Class 3, moderately diseased—more than one-half of leaves dead and remainder yellowing and wilting, often unilaterally, except at the green growing tip; Class 4, dead—dead plant tip.

Disease indices were calculated for each row of each treatment by the method used by Walker and Foster (7).

EXPERIMENTAL RESULTS

Disease development curves for Delwiche Commando and Wisconsin Perfection in control rows and in rows inoculated 8 days after planting by the dip and clip methods at sand temperatures of 18°, 21°, 24°, and 27° C. are shown in figure 1. These data represent arithmetical averages of disease indices obtained from duplicate tests at each temperature. At 18°, Wisconsin Perfection inoculated by the clip method showed a more rapid and more severe disease development than when inoculated by the dip method. Near-wilt resistant Delwiche Commando in both inoculated and check rows, and Wisconsin Perfection in check rows, showed a much lower and nearly similar amount of disease development. It may be seen in figure 2 that the Wisconsin Perfection seedlings in rows 4 and 8 inoculated by the clip method were more severely diseased at 20 days than those in row 2 inoculated by the dip method. However, the differential between the resistant and susceptible types of reaction at this temperature in this length of time was not so great as was desired.

At 21° the rates of disease development were in much the same relative order as at 18°, though they were generally more rapid and advanced at the end of 20 days. Wisconsin Perfection seedlings inoculated by the clip method had almost completely succumbed to the disease in from 15 to 20 days following inoculation, while resistant Delwiche Commando, in a like period of time, showed disease indices of 7 in 15 days to 30 in 20 days following inoculation. The clip method of inoculation was superior to the dip method for obtaining a differential for reaction to near-wilt at this temperature without a significant breakdown of resistance of Delwiche Commando. The comparison is evident in figure 2, where rows 3 and 4, 7 and 8, inoculated by clipping, show a better disease reaction differential than rows

1 and 2, inoculated by dipping. The control rows appeared little affected by the clipping treatment using distilled water.

At 24° the rates of disease development retained the same relative order as at 21°, although they were more rapid. Wisconsin Perfection seedlings inoculated by the clip method gave, at 18 days after inoculation, an average disease index of 100, while at 20 days those seedlings inoculated by the dip method did not exceed a disease index of 82. The resistance of Delwiche Commando, however, had begun to break down, since at 24°, by the clip method, it attained a disease index of 36, which was slightly higher than the index value at 21°.

At 27° C., the rates of disease development for the inoculated seedlings were considerably faster than at the lower temperatures. Disease indices for Delwiche Commando, for the clip and dip methods of inoculation, rose to 66 and 59 respectively after 20 days, indicating a continued decrease in disease resistance at higher temperatures. The appearance of Delwiche Commando plants at 27° C., 20 days after the two types of inoculation, is shown in figure 2. At this temperature the distinction between resistant and susceptible plants was obscured by the aggressive action of the pathogen.

In early experiments, seed coats were removed from the seed at time of inoculation. Decay of many of the seed coats provides a medium for the growth of the near-wilt organism and enables it to enter and rot the cotyledons. When this occurs it is difficult to distinguish between plants in the resistant and susceptible classes. An experiment was planned to determine whether seed protectants could be used in the near-wilt test to reduce seed decay before and after inoculation. The materials used were tetrachloroquinone (Spergon) and sodium hypochlorite (Clorox). A solution was prepared by mixing one part Clorox with 10 parts of water. Seeds were first immersed in a solution of Dreft, a commercial detergent, washed in two changes of distilled water, immersed in the Clorox solution for 10 min., and then washed free of the treatment solution with three changes of distilled water. For the Spergon treatment, seeds were shaken in a small bottle with a sufficient quantity of the protectant dust to completely cover them with an amount in excess of that used commercially.

Two 6-in. clay pots were provided for each treatment except the control. The drainage holes were covered with 1-in. square pieces of glass wool and the pots were filled with sterile white quartz sand to within about 1½ in. of the top. Six seeds of each variety were planted per pot, one variety on each side. Eight days after planting, the seedlings were removed and the seeds examined for decay. Seed coats were removed from half of the seeds in each treatment. Plants intended for inoculation were then given the clipping treatment as previously described. This experiment was conducted in an air temperature sufficiently high to maintain a sand temperature of 21° to 23° C.

Data from the seed treatment experiment are summarized in table 1.

During the 9 days after planting and preceding inoculation, none of the 46 seeds treated with Spergon showed decay of the seed coat. Seven seeds, however, had minor cotyledonary lesions apparently originating from invasions of fungi before the seed had been harvested. Clorox was less effective than Spergon, for 12 seeds out of 46 that were examined had decaying seed coats and 3 had minor cotyledonary lesions. Twenty-seven out of 48

TABLE 1.—*Effects of seed treatments upon seed decay and disease index of near-wilt resistant Delwiche Commando and near-wilt susceptible Wisconsin Perfection peas*

Treatment	Variety[a]	Type of seed infection at inoculation time		Total plants	Disease index after indicated days from date of inoculation	
		Decaying seed coats	Cotyledonary lesions		20	27
		Number	*Number*	*Number*		
Seed coats left on						
Inoculated						
Clorox	DC	0	0	12	45	78
	WP	3	0	10	93	100
Spergon	DC	0	1	12	4	13
	WP	0	0	12	73	94
Control	DC	6	0	12	14	55
	WP	8	0	12	94	100
Noninoculated	DC	1	2	4	0	0
	WP	2	2	6	0	0
Seed coats removed						
Inoculated						
Clorox	DC	4	1	12	11	25
	WP	5	2	12	77	94
Spergon	DC	0	3	11	9	9
	WP	0	3	11	63	88
Control	DC	4	0	12	17	36
	WP	9	1	12	88	100
Noninoculated	DC	3	1	6	0	0
	WP	3	0	5	0	0

[a] DC = Delwiche Commando; WP = Wisconsin Perfection.

untreated seeds had decaying seed coats and only one of these had a cotyledonary lesion. It was clear that Spergon effectively protected the pea seeds from attacks by fungi during the period from planting to time of inoculation.

Disease indices of 78 and 55 at 27 days were obtained for Delwiche Commando, treated with Clorox and in the control, respectively, in comparison with the much lower disease index of 13 for Commando given the Spergon treatment. Spergon prevented the resistance breakdown that results from invasion of the cotyledonary region by the near-wilt fungus following inoculation when the seed coats are not removed. Further evidence for the rôle of the fungus in attacking unprotected cotyledons and inciting disease is

the fact that no near-wilt developed in noninoculated Commando plants produced from seeds with seed coats in place, even though such seedlings had signs of decay 8 days after planting. The susceptible Wisconsin Perfection grown from Spergon-treated seed gave an index of 94 which was six points lower than for the other two treatments in the series where seed coats were left intact. The greater differential between the two varieties in the Spergon treatment appeared to be adequate in tests for reaction to near-wilt.

Table 1 also shows disease development indices for the three seed treatments when seed coats were removed from the seed at time of inoculation. Indices for the Spergon and check treatments are of primary interest. For the Spergon treatment the indices were 9 and 88 for the two varieties, a differential about the same as that in the series where seed coats were not removed.

An idea of the effectiveness of the near-wilt inoculation procedure accompanied by seed treatment can be gained from a sample of the results obtained in actual breeding studies. Forty-six F_3 lines harvested in bulk in 1947 were tested in the F_4 generation. This cross had been tested in F_2 in the field in 1946 and the susceptible plants discarded. In the greenhouse test, 29 of the lines were classified as segregating and 17 as resistant. This was a very close approach to the expected 2:1 ratio.

DISCUSSION AND SUMMARY

The object of the investigation was to develop a standardized technique whereby breeding material could be tested in the greenhouse for reaction to the near-wilt disease, incited by *Fusarium oxysporum* f. *pisi* (Linford) race 2 Snyder and Hansen. The experiments demonstrated that uniform and consistent results could be obtained by planting seedlings in clean quartz sand and inoculating 7 to 10 days later by cutting the roots about 1 in. below the seed while they were immersed in a spore and mycelial-fragment suspension; 18 to 20 days later an adequate index of reaction to near-wilt was obtained. This test was more rapid than when peas were planted in the greenhouse in field soil infested with the near-wilt organism. Under the latter conditions it was shown by Virgin and Walker (4) that severe disease development occurred only after 52 days from the time of planting. The former test was more dependable than disease reaction trials made in the field where environmental conditions were often unfavorable for the complete elimination of susceptible plants.

A sand temperature of 21° C. maintained throughout the period of testing was found to provide the best differential between the resistant and susceptible varieties. At 24° the resistant variety showed greater disease development, while at 27° the breakdown of resistance was still more pronounced. At 18°, disease development was so slow that after 18 to 20 days it was not possible to differentiate accurately between resistant and susceptible plants.

It was shown that if seed is treated with Spergon before planting it is unnecessary to remove the seed coats at the time of inoculation.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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EFFECT OF SOIL MOISTURE AND MINERAL NUTRIENT CONCENTRATION ON THE DEVELOPMENT OF BEAN POWDERY MILDEW

C. E. Yarwood

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The observation that some powdery mildews develop more luxuriantly in dry seasons and situations than under relatively wet conditions has been recorded by a competent authority as early as 1864 (1). In more recent times Cook (3) observed that bean mildew (*Erysiphe polygoni* DC) developed luxuriantly on plants in dry soil, and Volk (10) showed, in controlled soil moisture tests, that *Erysiphe graminis* developed more and caused less host necrosis on rye grown in soil at 40 per cent of its water-holding capacity than in soil at 60 or 80 per cent of its water-holding capacity. Nevertheless, much contrary opinion exists, and a recent plant pathology text states that powdery mildew of cereals "is most severe under moist conditions and those favoring vigorous, luxuriant growth of the host" (2). So far as the writer is aware no quantitative information on the effect of soil moisture on the injury caused by any powdery mildew has been presented, and this report on the subject may therefore be of value.

METHODS

For soils in which plants are growing there is no satisfactory method of maintaining soil moisture at uniform levels below field capacity (6). When an attempt is made to wet a soil mass to some moisture content less than field capacity, a portion of the soil is wetted to field capacity and the remainder is not penetrated, or penetrated very slowly, by the added moisture. Therefore problems of the type described here, and similar ones, might be considered insoluble by conventional methods. Nevertheless, conditions resulting from varying additions of water below the field capacity of the root-bearing mass bring about changes in plant growth and in plant disease development which are economically important and worthy of description, and seem most appropriately ascribed to soil moisture.

For soil moisture studies a uniform group of 5-in. pots was tared with well-mixed loam soil, planted with 5 Pinto bean seeds per pot, and watered uniformly until the plants were up, about 7 days after seeding. The plants were thinned to 2 per pot, the gross weight of the pots for moisture saturation to field capacity was determined, and the pots were divided into 2 to 5 groups for varying soil moisture conditions from field capacity to a little above the permanent wilting point. Enough water was added to the soil surface of each pot each morning to bring the gross weight to that arbitrarily decided upon. For each soil moisture condition one set of plants was inoculated with powdery mildew by spraying both leaf surfaces with a sus-

pension of conidia in water, and another set was maintained as a control. If controls were maintained longer than 2 weeks it was necessary to destroy on them the mildew that resulted from natural infection. For this purpose 0.1 per cent lime sulphur solution was sprayed on the plants. This treatment had little effect on the growth of non-mildewed plants. At the conclusion of the test, soil moisture samples were taken and the plants were harvested. One soil sample for each moisture condition was taken in the morning before the pots were tared with their daily addition of water, and another after. Samples of about 20 grams, taken after the entire soil mass in a given pot was well mixed, were dried in an oven for 3 days at 90° C. and weighed. The moisture present was designated in percentage by weight of the oven dry weight of the soil. In this way the approximate maximum daily range in soil moisture for each moisture condition was determined for most trials. An attempt was made to determine the permanent wilting point of the soil in one trial. Six pots of bean plants grown under conditions of regular and adequate watering were held without water for 3 days, and all plants were severely wilted. When pots were placed in a moist chamber, plants in 3 of the pots recovered their turgor in 24 hr. but plants in 3 pots remained wilted. Soil samples contained 7.3 to 11 per cent moisture where recovery occurred and 4.7 to 8.6 per cent where plants were permanently wilted. The permanent wilting point of this soil, therefore, was close to 7 per cent, which was about 25 per cent of the water-holding capacity. This wilting point is considerably above the lowest soil moisture recorded for plants growing in the driest soil. Soil samples were taken after the soil was mixed and the recorded soil moisture in the low soil moisture series would be the average of the air-dry soil in the bottom of the pot and the smaller amount of moist soil in the upper part of the pot.

For studies of the effect of nutrient concentration on the development of bean mildew, Pinto beans were seeded in sand and later transferred to 300-cc. flasks of water or Hoagland's solution (5).

For green weight yield, all leaves were weighed, and in some trials the weight of stems beyond the primary leaves was included because this stem growth had formed after treatments were begun. Time of harvest varied in order to secure the greatest effects of mildew infection and of soil moisture or nutrient treatment in the shortest time, but was usually about 6 weeks after seeding.

Reduction in yield due to mildew infection was calculated as the difference between the green weight of the healthy and mildewed plants expressed as a percentage of the green weight of the healthy. This method indicates much greater yield differences due to treatment than would be apparent from absolute values. For example, in the data presented in figure 1, the absolute reductions in yield due to mildew infection at low and high soil moisture were 1.50 and 1.47 gm., respectively, an insignifi-

cant difference, while the percentage reductions were 81 and 39, respectively, a highly significant difference.

Mildew development was rated on an arbitrary scale of 0 to 10 in which 0 indicated no mildew apparent, and 10 indicated that the leaf was entirely covered with a vigorous growth of mildew. In some trials mildew infection was measured quantitatively as conidia produced per unit area per day, but this method was not very satisfactory. The day prior to such an examination most of the conidia were removed by shaking the leaves. The next day slides were pressed against typical mildewed areas and the number of conidia per low-power field of the microscope was counted. Since the efficiency of the method was not tested, it is likely that the number of conidia found was less by some undetermined amount than the number actually present. Host necrosis due to mildew infection was rated on an arbitrary scale of 0 to 10 in which 0 indicated no necrosis apparent to the unaided eye and 10 indicated that a great majority of epidermal cells of upper leaf surface were necrotic. Host necrosis following powdery mildew inoculation is a common manifestation of resistance.

EXPERIMENTAL RESULTS

Results of a typical trial of the effect of soil moisture on yield and mildew severity are summarized in part in figure 1. There were 2 plants per

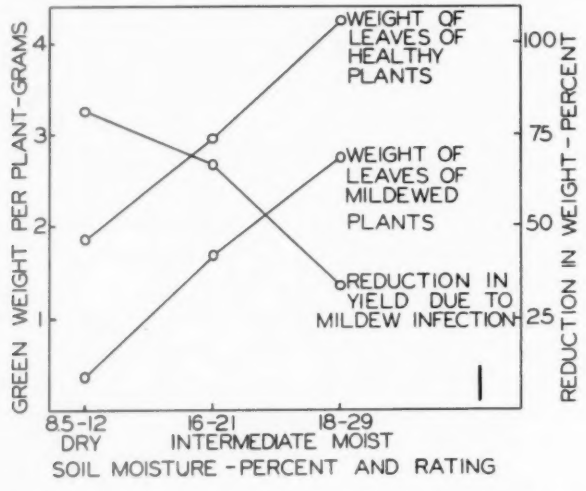

FIG. 1. Effect of soil moisture on the green weight of leaves from healthy and mildewed plants, in one trial started in November and harvested December 18, 1946.

pot and 4 pot replications of each treatment. The seed was sown on November 9, 1946, soil moisture adjustments at 3 soil moisture levels were started November 19, half the plants were inoculated November 27, and the plants were harvested and soil moisture samples taken December 18. Plant growth increased greatly with increasing soil moisture. The green

weight per plant of healthy plants was 1.83 gm. at low soil moisture, 2.96 gm. per plant at intermediate soil moisture, and 4.22 gm. per plant at high soil moisture. Mildew developed luxuriantly and without causing host necrosis on the small dark green primary leaves formed at low soil moisture; it was progressively poorer and host necrosis was extensive on the large pale leaves formed at intermediate and high soil moisture. Reduction in yield due to mildew infection decreased with increasing soil moisture, being 82 per cent in dry soil at 8.5 to 12 per cent moisture, 67 per cent in soil at 16 to 21 per cent moisture, and 39 per cent in soil at 18 to 29 per cent soil moisture, when the water-holding capacity of the soil was 29 per cent of its oven-dry weight.

Average results of all 16 trials of the effect of soil moisture on bean powdery mildew are summarized in figure 2. The number of soil moisture

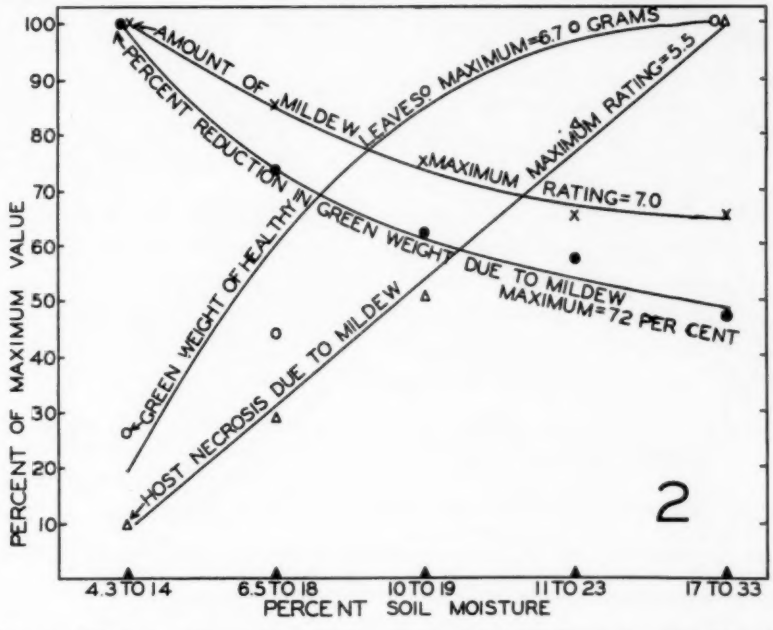

FIG. 2. Effect of soil moisture on the green weight of leaves from healthy plants, on the amount of mildew on inoculated plants, on the reduction in green weight due to mildew infection, and on host necrosis due to mildew infection.

gradations, the number of replications, the age of the plants at the time of soil moisture adjustments, the dosage of inoculum, the time of year, the age of infection at time of recording results, and the completeness of the record, all varied in different trials and are believed to have an important though inadequately determined bearing on the results. In other words, the slopes of the curves in figure 2 would be and were greatly different under different conditions. For example, the amount of mildew and host necrosis was greatly affected by the amount of inoculum and age of the leaves at time of inoculation. Although infection has been secured from

single conidia, infection resulting from scanty inoculum was relatively weak and necrosis due to mildew was relatively severe on leaves grown at high soil moisture. If, on the other hand, leaves were inoculated heavily while young, the difference in mildew development due to soil moisture was relatively slight. Such differences, as well as those due to nutrient concentration, were greater on primary bean leaves than on secondary bean leaves.

At time of harvest the bean roots had extended throughout the 5-in. depth of the soil layer, and even in the low moisture series where the lower layers of soil were air-dry these lower roots were still turgid, indicating that they might still be functioning and that they were receiving moisture from the regularly watered roots above. It is not known what bearing, if any, these turgid roots in dry soil had on mildew development.

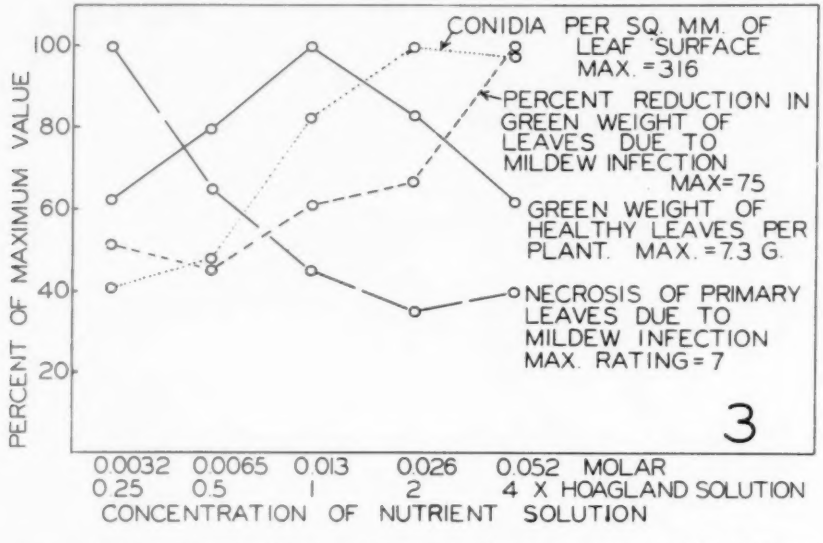
CONIDIA PER SQ. MM. OF LEAF SURFACE MAX. = 316
PERCENT REDUCTION IN GREEN WEIGHT OF LEAVES DUE TO MILDEW INFECTION MAX=75
GREEN WEIGHT OF HEALTHY LEAVES PER PLANT. MAX. = 7.3 G.
NECROSIS OF PRIMARY LEAVES DUE TO MILDEW INFECTION MAX. RATING = 7
3
PERCENT OF MAXIMUM VALUE
100
80
60
40
20
0.0032 0.0065 0.013 0.026 0.052 MOLAR
0.25 0.5 1 2 4 X HOAGLAND SOLUTION
CONCENTRATION OF NUTRIENT SOLUTION

FIG. 3. Effect of concentration of nutrient solution on green weight of leaves of healthy plants, on the reduction in green weight due to mildew infection, on the production of conidia, and on host necrosis due to mildew infection.

The plants growing in soil at the lowest moisture content frequently wilted during the day and remained wilted until the regular watering next morning. This wilting did not seem to be detrimental to the mildew growing on these leaves, and may perhaps have favored it, though this was not studied. Plants growing in the next to the lowest soil moisture condition (6.5 to 18 per cent) also wilted on bright days. Plants at the 10 to 19 per cent soil moisture level were never observed to wilt, yet they yielded less than plants at higher soil moisture, indicating that soil moisture levels below field capacity but above the wilting point influenced plant growth.

Results of three trials to determine the effect of mineral nutrient concentration on the development of bean powdery mildew are presented in figures 3 and 4. Increasing or decreasing the nutrient concentration in the

Hoagland formula decreased the growth of beans. Mildew development and reduction in yield due to mildew increased with increasing nutrient concentration while necrosis due to mildew decreased. In two other trials mildew severity on the primary leaves of plants grown in distilled water and 0.1, 0.2, and 10 times the concentration of nutrients in the Hoagland

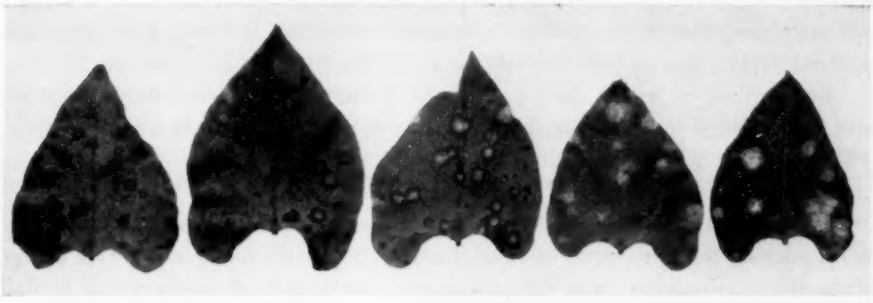

FIG. 4. Effect of nutrient concentration on the development of powdery mildew on primary bean leaves. From left to right the primary leaves are from plants growing in 0.25, 0.5, 1, 2, and 4 times the concentration of nutrients in Hoagland's formula. Mildew development is progressively greater, and host necrosis due to mildew is progressively less from left to right.

formula showed a similar relation, but these nutrient conditions were all beyond the limits for good growth of beans. These results with bean mildew appear in accord with those of Grainger (4) for oat mildew.

From plants for these trials grown in water culture and in soil, data on the dry matter content of the leaves and on nodulation of the roots were secured and average results are presented in table 1. In both water and

TABLE 1.—*Effect of cultural and infection conditions on dry matter content of leaves and nodulation of roots*

Concentration of nutrient solution or soil moisture content	Percentage dry matter of leaves		Root nodules per plant	
	Healthy	Mildewed	Healthy	Mildewed
A. Plants in water culture				
0.0032 molar	13.0	13.5	59	28
0.0065	12.5	13.0	16	8
0.013	11.5	11.9	9	2
0.026	12.7	13.1	2	0
0.052	15.5	16.9	0	0
B. Plants in soil				
10.2 per cent soil moisture	10.4	11.3		
18.5	8.5	9.5		
23.5	7.5	9.2		

soil cultures mildewed leaves always had a greater percentage of dry matter than healthy leaves. The mildewed plants always yielded less than the healthy (Figs. 1, 2, 3). The percentage of dry matter of healthy leaves from plants grown in water culture decreased with increasing nutrient con-

centration up to 0.013 M and then increased up to 0.052. Plant growth was greatest at 0.013 M (Fig. 3). With plants in soil the percentage of dry matter of healthy leaves increased from 7.5 to 10.4 per cent as soil moisture decreased from 23.5 to 10.2 per cent. Plant growth was greatest at 23.5 per cent (Fig. 1, 2). Therefore with respect to infected *vs.* healthy plants, low *vs.* high concentration of nutrient solution, and low *vs.* high soil moisture content there was a positive correlation between high moisture content of the leaves and growth vigor of the plants.

Nodulation of roots decreased with increasing nutrient concentration and was always lower for mildewed than for healthy plants (Table 1).

OBSERVATIONS ON OTHER POWDERY MILDEWS

In addition to the studies of bean powdery mildew already reported, the effect of soil moisture was studied for the powdery mildews of red clover (*Erysiphe polygoni*), pea (*E. polygoni*), mustard (*E. polygoni*), barley (*E. graminis*), cucumber (*E. cichoracearum*), sunflower (*E. cichoracearum*), rose (*Sphaerotheca pannosa*), and grape (*Uncinula necator*) in the greenhouse at Berkeley, California, and for the powdery mildews of bean, barley, cucumber, sunflower, and rose in the field. Of these powdery mildews, all with the exception of grape powdery mildew developed better on plants at low soil moisture than on plants at high soil moisture, but the effect was pronounced only with bean and with mustard. With beans the varieties Pinto, Scarlet Runner, and Dwarf Lima were used in one parallel test and the favorable effect of low soil moisture on mildew development was most pronounced on Pinto. With barley the effect of soil moisture on mildew development was tested for the varieties Atlas, Black Hulless, Kwan, Algerian, and Psakwan, a series ranging from high susceptibility to high resistance. It was thought that low soil moisture might greatly lower the genetic resistance of the resistant varieties, but such was not the case. The apparent greater mildew infection on grape at high soil moisture than at low soil moisture was slight in the only test performed and is not considered significant.

DISCUSSION

The basic reason why beans and some other plants grown under conditions of low soil moisture or high nutrient concentration are more susceptible to powdery mildew than plants grown under conditions of high soil moisture or low nutrient concentration is not known. The writer believes that both situations may be related to the xerophytic nature of powdery mildews (11), since conditions which favor mildew development are those producing a xerophytic environment for the plant. Here, in contrast to the previously described situation (11), however, the region of treatment—*i.e.*, the roots, is far removed from the region of response, the leaves. The greater water content of leaves from plants grown in 0.013 M nutrient solution than of leaves from plants grown at lower or higher nu-

trient concentration, while mildew increased progressively from 0.0032 to 0.052 M, would not support this point of view. Of course there is a nice correlation between supply of nutrients to the host and mildew development, and this may be the proper explanation. Opposed to this argument is the fact that increasing nutrient concentrations beyond 0.013 M which increased mildew development did not increase plant growth.

Although the opinion popularly prevails that powdery mildews, like obligate parasites in general, develop more luxuriantly on a vigorous than on a weak host (7), little support for it is found in this study. Leaves on plants grown at low soil moisture were much darker green and more susceptible to mildew than leaves of plants grown at high soil moisture. However, for plants in soil there was an inverse relation between mildew development and host growth (see figure 2), and for plants in water culture there was a direct relation between mildew development and plant growth below 0.013 molar nutrient concentration, but an inverse relation above 0.013 molar. A similar lack of correlation between wheat growth and wheat mildew development has been reported by Trelease and Trelease (9) in studies of the relation of mineral nutrition of the host to mildew development.

The possibility that the treatments to which the plants were subjected affected the relative humidity at the leaf surfaces, which in turn affected mildew development, is considered unlikely though the effects are partly in accord with the idea that powdery mildews may be favored by low atmospheric humidity (11). Perhaps the expected lower turgor pressure of the leaves on plants grown at low soil moisture or high nutrient concentration is involved, since Rivera (8) has reported that decreased turgor increased the susceptibility of wheat to powdery mildew.

SUMMARY

On Pinto bean plants grown in a series of soil moisture levels from 4 to 33 per cent of the dry weight of the soil, mildew developed more luxuriantly, host necrosis due to mildew was less, and reduction in green weight due to mildew infection was greater at low than at high soil moisture. Powdery mildews of red clover, pea, mustard, barley, cucumber, sunflower, and rose in greenhouse and field trials also developed more luxuriantly on plants grown at low than at high soil moisture, though quantitative results were not taken in most cases. On beans grown in water culture with nutrient concentrations ranging from 0 to 10 times the concentration of nutrients in Hoagland's solution, mildew development increased, host necrosis due to mildew decreased, and the effect of mildew on green weight increased with increasing nutrient concentration, while plant growth was greatest at the standard concentration.

DIVISION OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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LIFE HISTORY AND CONTROL OF THE ASCOCHYTA RAY BLIGHT OF CHRYSANTHEMUM

KENNETH F. BAKER, A. W. DIMOCK, AND LILY H. DAVIS[1]

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The Ascochyta ray blight of chrysanthemum (*Chrysanthemum morifolium* Ram.) was first observed by F. L. Stevens outdoors and under glass in two counties of North Carolina during November, 1906, and the pathogenicity of the fungus, *Ascochyta chrysanthemi* Stev., was established (16). The disease apparently had been known there since 1904. According to records of the Plant Disease Survey,[2] it was reported there again by Stevens in 1907 and by H. R. Fulton in 1913. In South Carolina it was reported by H. W. Barre in 1912 and 1913 and by R. F. Poole in 1931. In Mississippi it was reported by L. E. Miles in 1932 (21). The disease occurred in outdoor plantings in Maryland in 1947 and was severe in 1948, causing typical flower blight as well as stem cankers.[3] The occurrence of the disease in Florida has recently been confirmed by us. Apparently the fungus has continued to be destructive in the area of its first recognition in North Carolina, and caused severe losses in some field and cloth-house plantings in 1946 and 1947.

H. N. Hansen and W. C. Snyder observed Ascochyta pycnidia and Mycosphaerella perithecia on dead stem bases of commercial chrysanthemums from San Mateo county in March, 1949. Single-ascospore and single-conidium cultures proved these to be the same fungus. Single-conidium cultures grown on plain agar plus cold-sterilized pea stems developed mature perithecia in 10 days outdoors. Flower isolates produced typical petal spots which then advanced into ray blight. These results with the California isolate have been confirmed by us at Los Angeles. Pycnidia and perithecia developed on petals of flowers exposed under moist outdoor conditions for 13 days. The disease has never been reported in California and this record is therefore of unusual interest since it shows that the fungus will persist in an area of unfavorable climate where the natural flower infections are not known to occur.

The fungus was mentioned in publications from Ohio (13, 19), Missouri (12), New Jersey (11), New York (3, 7), Wisconsin (1), Colorado (4), and Germany (10), apparently based on literature review and without any actual evidence of its occurrence in these areas.

[1] The careful observations and generous assistance of Mr. Leo C. Gould, Pittsboro, North Carolina, is acknowledged with pleasure.

[2] Letter of May 11, 1948, from Dr. Freeman Weiss.

[3] Letter of December 18, 1948, and photographs from Dr. L. O. Weaver. Specimens had been identified by Dr. W. F. Jeffers and Dr. J. A. Stevenson, and the identity confirmed by us in comparative inoculations with the North Carolina fungus.

FIG. 1. Ascochyta ray blight of chrysanthemum. A, naturally infected flowers (Pittsboro, North Carolina, September, 1947) showing characteristic unilateral infections. B, experimental conidial infections on moderately susceptible Goldsmith after 96 hr. Enlarged petals below. C, same on very susceptible Arcadia. Detached petals half-rotted. Photographs by W. R. Fisher, Department of Plant Pathology, Cornell University.

The present studies were conducted to clarify some points in the life history of the fungus, to determine whether there was danger that it might become established in other areas, and to devise control procedures for the disease produced.

SYMPTOMS

Symptoms in naturally infected material were found to agree with those described and illustrated by Stevens (16). The disease is first noted and is most important in its attack on the ray flowers of the head. Infection usually is initiated on one side of the blossom but may later spread until all the florets are involved (Fig. 1, A). Growth of the fungus char-

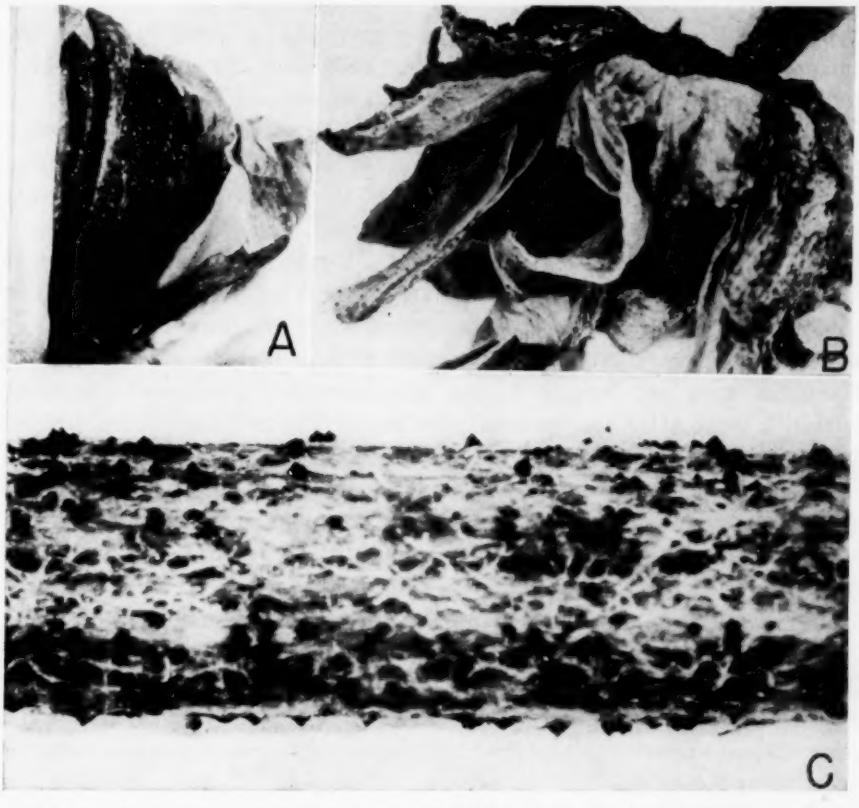

FIG. 2. Sporulation of Ascochyta ray blight fungus on chrysanthemum. A, enlarged Ascochyta pycnidia on petals of flowers from Pittsboro, North Carolina, September, 1947 (×5). B, enlarged perithecia on flower inoculated with conidia (×4). C, enlarged perithecia on stem of naturally infected material held in the laboratory (×16). Photographs by W. R. Fisher, Department of Plant Pathology, Cornell University.

acteristically causes tissue discoloration of the floret progressively upward from the receptacle. The affected petals turn tan to light brown and remain so except when invaded by secondary organisms. In many, perhaps most, cases the fungus grows down into the peduncle for several centimeters, causing it to turn black and weaken, and the head to droop. The

fungus frequently attacks unopened buds and their peduncles, darkening the bracts and stem tissue.

Leaf infection is fairly common on plants having diseased flowers, producing in the lamina irregular black blotches up to 2–3 cm. across. Black girdling lesions several centimeters long frequently are noted on the stems, usually starting at a node.

Pycnidia were abundant in all affected tissues of most field infections seen by us, particularly in the petals (Fig. 2, A). These structures, amber-colored at first but later darkening, were observed readily with a 15× hand lens. It also was noted in our field material that the infected florets, particularly those bearing pycnidia, were almost invariably stuck together. Whether the petals were bound together by the permeating hyphae or were cemented by the gelatinous material extruded with the spores was not determined, although the following observations suggest the latter.

Certain additional symptoms became evident on material inoculated with suspensions of either spores or mycelial fragments and maintained under laboratory temperatures of 22°–24° C. The first evidence of infection was the appearance of abundant, minute, deep pink spots[4] on the petals of the test varieties Arcadia, Rev. Bushnell, and Goldsmith (Fig. 2, B). Within a few hours the petals of Arcadia were badly rotted, becoming tan in color. Although overgrown with mycelium when kept under a bell jar, they remained distinct rather than becoming stuck together. Pycnidia were not formed in the laboratory but developed abundantly within a week after the material was exposed to the fluctuating temperature of the laboratory roof (Ithaca, N. Y., in April).

Leaves inoculated with suspensions of spores or mycelial fragments in the laboratory soon developed abundant, minute, black lesions. The surrounding green tissues yellowed very rapidly when the leaves were maintained in a moist chamber. Although the lesions enlarged somewhat, blotches similar in size to those on field material were not formed.

The disease may develop with dramatic rapidity under rainy conditions in the field, causing total loss of entire blocks of susceptible varieties which appeared perfectly healthy several days previously. Symptoms may appear also on apparently healthy cut blooms in transit to market, rendering them unfit for sale.

The disease is distinguished easily from all other chrysanthemum troubles. *Botrytis cinerea* Pers. may cause tiny brown spots and finally produce decay of the flowers under cool humid conditions (15), but generally the characteristic gray sporulation occurs copiously on the petals. Usually Ascochyta-infected flowers are free from secondary fungi, and the pycnidia are conspicuously abundant. *Septoria* spp. are not known to attack chrysanthemum petals but are common on leaves, producing symp-

[4] This symptom was also reported by a commercial grower as the first evidence of field infection.

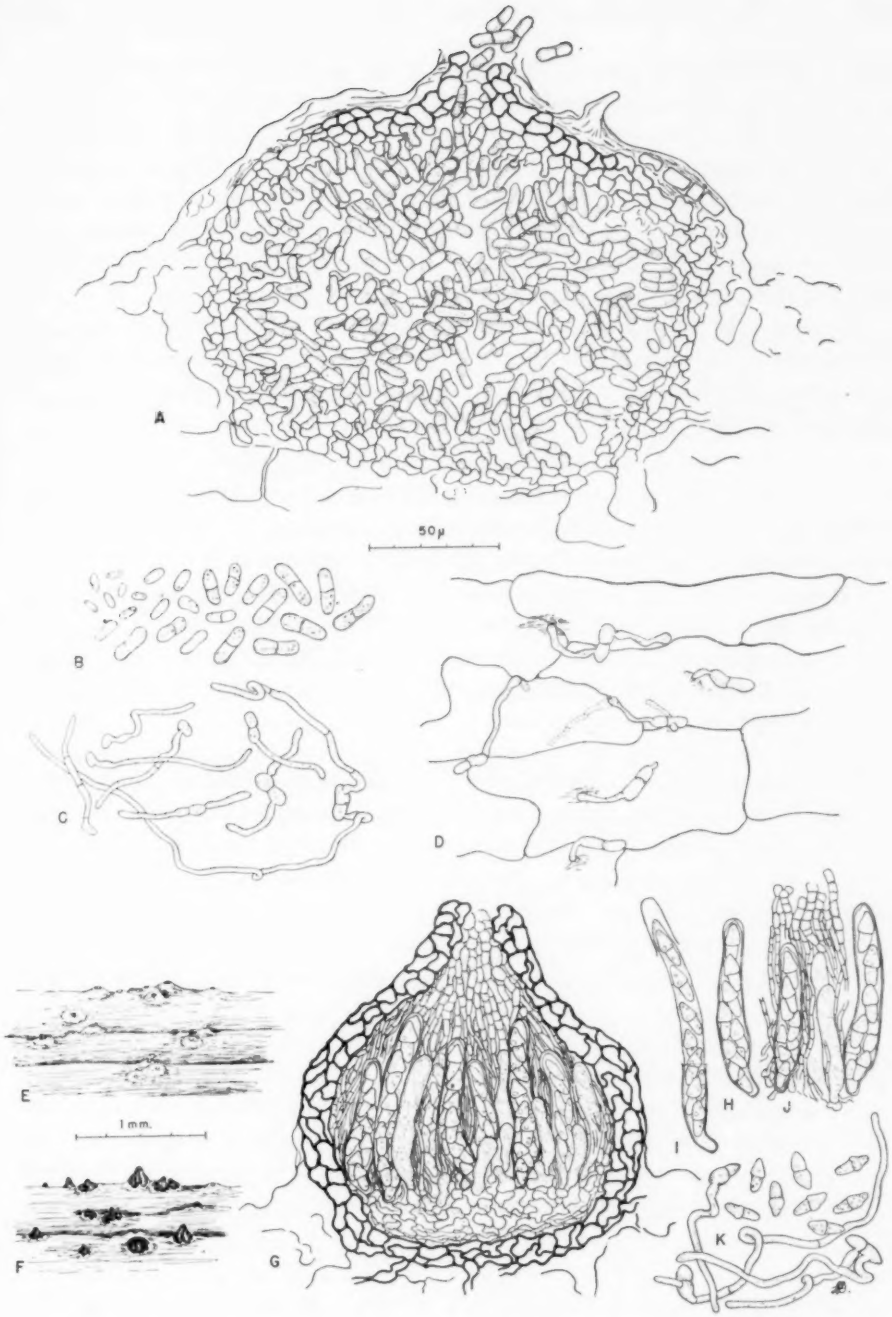

FIG. 3. Camera lucida drawings of *Mycosphaerella ligulicola*. Magnification of A–D and G–K on the 50 μ scale. A, cross section of pycnidium of Ascochyta stage. B, conidia, ranging from microspores to uniseptate type. C, germinating conidia on water agar after 18 hr. D, composite of several germinating conidia penetrating chrysanthemum petal, 7 to 23 hr. after inoculation. E, habit sketch of pycnidia on peduncle, showing oozing spores. F, habit sketch showing perithecia on stem. G, cross section of perithecium showing asci in various stages of maturity, and strands of interthecial tissue. H, mature ascus. I, elongated ascus, showing dehiscence. J, segment of asci and interthecial tissue. K, mature ascospores, and stages in germination after 15 hr. Drawings by L. H. Davis, Division of Plant Pathology, University of California, Los Angeles.

toms somewhat different from those caused occasionally by *Ascochyta*. The margins of Septoria spots are more definite than those of *Ascochyta*, and the central areas have a characteristic sheen. Leaf tissue surrounding Ascochyta spots turns yellow more commonly than does that surrounding Septoria spots. The pycnidia of *Ascochyta* usually are pale and inconspicuous, with a darkened ostiole from which spores exude in amber drops or short tendrils (Fig. 3, E); those of *Septoria* are conspicuous and black, and exude spores in long white cirri.

Because the most characteristic symptom is the blighting of the ray flowers, in which abundant pycnidia are produced, the most descriptive and appropriate common name for the disease is Ascochyta ray blight of chrysanthemum.

VARIETAL REACTIONS

Controlled tests of varietal susceptibility were not undertaken, but the following tentative ratings of varieties current in the trade were supplied by a commercial grower in North Carolina.

Resistant: Golden Herald, Hasagawa Gold, Irene, Minong, Nuggets, Red Daisy, Royal Crimson, Yellow Daisy, Yellow Irene.

Intermediate: Barcarole, Bittersweet, Bulkeley, Cameo, Capt. Cook, Carnelia, Golden Spray, Goldsmith,[5] Pink Dot, Pinocchio, Rev. Bushnell,[5] Robin Hood, Valencia.

[5] Confirmed in our laboratory tests.

Very Susceptible: Arcadia,[5] Caroline Yosick, Cassandra, Gold Coast, Jessie, Jewell, Lakme, Mary Lennon Hall, Masterpiece, Navaho, October Bronze, Pink Pearl, Pixie, Princeton, Priscilla, Sea Gull, Shasta, Silver Sheen, Sunnyside, Sylvanna, Vesper, White Mensa, Yellow Dot, Yellow Hammer, Yellow Fellow, Yolanda.

Laboratory tests have shown Goldsmith to be somewhat less susceptible than Arcadia (Fig. 1, B and C), although still disastrously affected in the field.

The older varieties Nellie Pockett and Golden Wedding were noted by Stevens (16) as very susceptible.

Flowers and leaves of *Chrysanthemum leucanthemum* L. were not successfully infected in several attempts at Ithaca, N. Y., and Los Angeles, Calif. Because this plant commonly grows as an escape near the Pittsboro, North Carolina fields, it was considered a possible additional host of the fungus.

INFECTION STUDIES

Infections were successful in every attempt, whether with conidia from infected tissue or cultures, mycelial fragments from nonsporulating cultures fragmented in a Waring Blendor, or ascospores from dead stems. Flowers of a very susceptible variety heavily inoculated (*e.g.*, Arcadia dipped in a dense conidial suspension) and kept moist under bell jars were completely rotted in 15 hr. Check blossoms dipped in clear water

remained healthy even though retained under bell jars for many days. Previously (16), infection had been demonstrated only with macerated cultures of the pathogen.

Time required for infection. Germination of conidia occurred on agar in 3½ to 4 hr., but petal infection was found to require somewhat more than 6 hr. Flowers of Arcadia were atomized with a heavy suspension of conidia from field-infected flowers, and placed under bell jars. At intervals up to 6 hr. some flowers were removed and dried for 1 hr. in the air-stream of an electric fan blowing over a hot plate, then maintained uncovered and dry in the laboratory. Earlier tests had shown that established infections would continue without maintaining the blossoms at high humidity. No infection was evident in any of these lots after 5 days, although abundantly present after 3 days on check flowers kept moist under a bell jar. When subsequently atomized with clear water and replaced under bell jars, all test lots developed infection, indicating that conidia may start germination, be checked by drying, and resume growth when wetted as much as 5 days later.

Temperature relations. The effect of temperature on the rate of petal infection was studied. Flowers of Arcadia were placed under bell jars in temperature cabinets for 24 hr., removed briefly to be atomized with a conidial suspension prepared from field-infected flowers, and immediately replaced in the chambers. Observations made 19 hr. after inoculation revealed a broad temperature range suitable for infection, extending from 9° to 26° C., as indicated by the presence of characteristic pink spots in the petals. After 24 hr., infection was observed at 30° C., and after 42 hr. very tiny spots were seen at 6° C. Thus petal infection occurred at 6°–30° C., with the 20°–26° C. range highly favorable, and the optimum at about 24° C. The lesions at 6° C. were barely visible even after 42 hr. The pink color reaction of this white variety was evident at 6°–30° C. The relative growth rate of the spots at the various temperatures paralleled the amount of infection which had occurred. No infections occurred at 38° C. and the flowers remained in good condition for several days. Some of these removed to laboratory temperatures after 42 hr. developed scattering typical pink spots in 2 days, indicating the survival of a low percentage of the conidia. The checks were not infected.

The ability of the fungus to withstand extremely low temperatures was shown by overwintering tests in which field-infected material was placed in wire baskets on the laboratory roof (Ithaca, N. Y.) on October 20, 1947. Conidia obtained from this material on March 24, 1948, were viable and produced abundant flower infection after this exposure to an extremely cold winter with official temperatures at a near-by weather station reaching −29° C. No ascospores were involved in this carryover. Similarly, material overwintered at Pittsboro, North Carolina, yielded viable conidia.

The fungus grew well on potato-dextrose agar at a wide range of labo-

ratory temperatures. The optimum was about 24° C., with excellent growth from 15° to 27° C., and fair growth at 6° C.

Manner of infection. Conidia germinate on the flower petals and develop short mycelia before penetrating directly through or between epidermal cells (Fig. 3, D). The characteristic much-branched, short-celled mycelium (16) quickly grows through the tissue intra- and intercellularly, causing a moist brown decay.

THE CAUSAL FUNGUS

The fungus investigated unquestionably was the same as that studied by Stevens (16), even though minor morphological variations were observed. The collections studied were from Pittsboro, North Carolina, about 50 miles from the type locality and 30 miles from Stevens' Raleigh report. Pathogenicity was established repeatedly by fulfilling Koch's postulates.

Pycnidia are produced abundantly on the infected petals under conditions of fluctuating temperature and moisture (Fig. 2, A) but are only rarely formed in a continuously moist environment. They are somewhat less abundant on stems or leaves. In potato-dextrose-agar cultures pycnidia commonly are not formed, but some single-spore isolates produced them copiously. In culture, the pycnidia are brown and frequently coalesce so that more than one ostiole occurs. They are highly variable in size, as are the spores. The latter are also either 1- or 2-celled (Fig. 3, B). The reported 3-celled conidia (16) were not seen by us.

On the host the pycnidia arise on petals, leaves, or stems directly beneath the epidermis. As they enlarge, the epidermis is pushed up, but does not rupture except where the neck pierces it. At maturity the pycnidium is completely immersed except for the short-necked pore (Fig. 3, A). The wall is thin, only 1 or 2 layers, and amber in color except at the neck, where the cells are dark brown and thicker. In many cases the basal wall consists only of a thin (single-celled), translucent, proliferous stratum of mycelium. On the stems, the pycnidia are usually scattered and somewhat larger than on the petals where they are small and aggregated, perhaps because of the faster withering of the petals.

The internal cavity of the pycnidium is filled with a mass of hyaline spores budded from the fertile cells of the inner wall. The short sterigmata that Stevens (16) illustrated as bearing the conidia were not observed by us. At first the small continuous conidia were thought to be spermatia, but they were found just as capable of germination and infection as the larger 1-septate spores. On the host the conidia ooze in short columns (Fig. 3, E), but when the pycnidium is immersed in a drop of water, long twisting tendrils are formed. As the spore takes up water in germination the cells become distended and more or less globose, so that the spore becomes very deeply constricted at the septum without increasing appreciably in length. Germ tubes arise from one or both cells (Fig. 3, C).

Monoconidial isolates of the fungus on potato-dextrose agar at laboratory temperatures formed an appressed feathery growth 1.7 cm. in diameter after 67 hr.; in 4 days the colonies were round, 2.7 cm. in diameter, and the growth was cottony white; by 6 days, the colonies averaged 5.2 cm. in diameter, were salmon pink beneath (because of a diffusible pigment), and dark brown in the center. The margins were irregular, white, and appressed, but toward the center the colony was rather dense, floccose, and bluish gray, later darkening. Mature pycnidia were found in the center of colonies of sporulating isolates, but toward the margins only white mycelial knots were formed.

Perithecia are present in large numbers on infected chrysanthemum stems, petals, or leaves (Fig. 2, B and C). They were first observed January 12, 1948, on petals of infected flowers from North Carolina that had been kept dry in the laboratory at Ithaca, New York, for about 8 weeks. During that period the humidity was extremely low, and there was no possibility of the stems having been wetted. Some inoculated flowers that had been held in a moist chamber until decayed and then dried in the laboratory failed to develop pycnidia, but when examined after 6 to 8 weeks mature perithecia were found. This extraordinary development of perithecia under essentially arid conditions has been repeatedly confirmed. Overwintered infected stems collected at Pittsboro, North Carolina, on June 8, 1948, were held for herbarium specimens in Los Angeles, California. There were a very few perithecia on occasional stems at time of collection, but when the stems were examined again in October there were many grayish patches in which mature perithecia were closely crowded. A conidial suspension was placed on flowers in a moist chamber in the laboratory (Los Angeles) on August 26, 1948. Twelve days later abundant mature pycnidia were evident on the drying tissues, and by the 18th day a few immature perithecia were noted on the dry stems. No record has been found of a fungus of this type producing its perithecia under such arid conditions, but similar maturation of the perithecia in the fall months has been observed (17, pp. 581–582, 584). Jones (6) reported production of mature perithecia of *Mycosphaerella pinodes* (Berk. and Blox.) Stone on living plants 13 days after inoculation.

Perithecia formed, but much less abundantly, in naturally infected stems and flowers exposed outdoors (Ithaca) during the winter. Such material was placed in metal baskets on the ground on October 20, 1947. By February 23, 1948, immature perithecia were present. During the period October 20–November 23, 1947, in which the daily maximum temperature on 28 days reached or exceeded 6° C. (the approximate minimum for growth of the fungus), the mean minimum was 1.1° C. and the mean maximum 12.2° C. From November 24, 1947 to February 23, 1948, when only 7 scattered days reached or exceeded 6° C., the mean minimum temperature was −11.2° C. and the mean maximum was −1.0° C.

The perithecia were shown to be part of the life cycle of the *Ascochyta* by culture and inoculation tests. Single-ascospore cultures gave the same fungus as that obtained from conidia. The particular isolates thus obtained did not produce pycnidia in culture, but when one of the cultures was fragmented in a Waring Blendor and atomized on blossoms of Arcadia chrysanthemums, typical spots and decay developed in which pycnidia were formed when exposed outdoors (Ithaca) for 3 days in early April. These flowers were then kept dry in the laboratory for 50 days and typical mature perithecia were produced (Fig. 2, C) in stems and leaves. This behavior and the corroborative evidence of Hansen and Snyder already mentioned indicate that this fungus is homothallic. In another test single perithecia were removed from stems that had developed no pycnidia, and were crushed in sterile water to give a suspension of asci and ascospores. When drops of this were placed on chrysanthemum flowers, typical spots and decay developed within a few days.

The perithecial initials apparently develop just beneath the epidermis as small stromatal knots of mycelium. These enlarge until they break through the epidermis, becoming so erumpent in some instances as to appear almost superficial, but are basally adnate (Fig. 3, F). The centrum is at first filled with a hyaline pseudoparenchymatous mass of tissue near the base of which a fascicle of asci develops. Typically, in most species of *Mycosphaerella*, the pseudoparenchyma is crushed and disintegrated as the asci grow up into it, but in this case it is only partially destroyed, the bulk of it remaining as interthecial tissue in the mature perithecium (Fig. 3, G and J).[6] In this respect the fungus approaches the Dothioraceae in the higher Myriangiales, where each ascus grows up in a separate stromal locule, and differs from the Mycosphaerellaceae which usually lack threads in the uniloculate stroma (8).[7] Although atypical for *Mycosphaerella*, such mycelial strands have been noted by various workers for fungi included in the genus (5, 17).

At maturity the perithecium consists of an outer wall of dark brown, thick-walled cells in 2 to 3 layers and a central cavity composed of several layers of crushed pseudoparenchyma at the base, from which arises a cluster of asci, each apparently enclosed between fine mycelial strands which join in the neck of the perithecium (Fig. 3, G and J).

Not all of the asci mature at once, so that all stages of maturation are observable in one perithecium. The mature ascus (Fig. 3, H) is obclavate, gradually narrowed above, and abruptly contracted at the base, thus being

[6] A special technique developed by L. H. Davis and J. G. Bald for rapid freehand sectioning under the wide-field microscope, and for prompt definitive staining of the sections has greatly facilitated these observations.

[7] Letter of July 24, 1948, from Dr. Julian H. Miller. After examination of a specimen of the chrysanthemum fungus, he classed it as an atypical *Mycosphaerella*. This concept is further discussed in the following paper: Miller, J. H. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia 41: 99–127. 1949.

somewhat stipitate. It contains 8 sub-biseriate ascospores. The wall is double, consisting of a thick, fairly rigid outer, and a thin, elastic inner membrane. When the ascus is sufficiently mature, it ruptures at a thin place in the middle of the ectoascus, thus releasing the endoascus which elongates and projects into the neck of the perithecium. The ectoascus sometimes remains as a thimble-like cap at the tip of the endoascus (Fig. 3, I). Although forcible discharge of ascospores was not observed, the structure and rupture of the ascus wall and the compression of the ascospores into a single row are evidence for this type of discharge. The ascospores germinate readily, each cell sending out a germ tube (Fig. 3, K).

Only one *Mycosphaerella* or *Sphaerella* appears to have been described on chrysanthemum. *Sphaerella Chrysanthemi* F. Tassi was reported on dead stems of *C. marginata* in Italy in October, 1900 (18, p. 117). This description is so indefinite as to fit many species of *Mycosphaerella*. The perithecial stage reported here has little morphological distinctiveness and it is unlikely, therefore, that anything more definite could be determined even by examination of Tassi's specimens, if they could be found. It is impossible to determine whether Tassi's fungus was the same as the one considered here, and the fact that the disease is not known outside of the United States suggests that his fungus was different. The destructiveness of the disease under a wide range of environmental conditions is such that some record of it on the extensively cultivated chrysanthemum would inevitably have appeared if the fungus was present in Italy. Tassi's record affords no evidence of pathogenicity of his fungus. If the name *M. chrysanthemi* were to be assigned to our fungus there would be an unwarranted implication that the disease it produces occurs in Italy. Because of these facts our fungus is considered to be distinct. The *Ascochyta chrysanthemi* Cav. cited by Seymour (14, p. 681) was found to be based[8] on a brief note by Selby (13, p. 388) and probably represents an erroneous carryover of the authority from *Septoria chrysanthemi* Cav. mentioned 11 lines above on the same page.

The fungus is here placed in *Mycosphaerella* instead of *Sphaerella*, in accordance with present general usage. The Nomenclature Committee of the British Mycological Society (20, pp. 220–222) has recommended that the name *Sphaerella* (Fr.) Rabenh. be conserved for the genus, but this name apparently is a later homonym of, and still is used for, an algal genus (9, pp. 78–79).

Because of the points cited, the perithecial stage is considered as new, and is here named *Mycosphaerella ligulicola* n. sp. (from Latin *ligula*, little tongue [botanically, ray flower], and *colo*, to inhabit).

Mycosphaerella ligulicola sp. nov.

Peritheciis in caulibus, foliis et ligulis mortuis dispersis vel aggregatis, subepidermide erumpentibus, maculis pallidis griseis formantibus, nigris, globulosis, 96–224 μ

[8] Dr. W. L. White, of the Farlow Herbarium, kindly supplied this information from Seymour's notes in a letter of January 10, 1949.

diam. (medio 146 μ), ostiolo papilliformi, pariete membranaceo; ascis octosporis, fasciculatis, obclavatis, utrinque rotundatis, breve stipitatis, 49–81 × 8–10 μ (medio 63 × 9 μ); ascosporis subdistichis, elliptico-fusoideis, 1-septatis, loculis inaequalibus, ad septum constrictis, hyalinis vel pallide viridulis, guttulatis, 12–16 × 4–6 μ (medio 14 × 5 μ).

Perithecia numerous, crowded on grayish spots on old dried stems, less frequently on petals and leaves; erumpent, becoming nearly superficial, globose, 96–224 μ in diameter (average 146 μ), with a shiny, black, membranous wall and a distinctly papillate ostiole. Asci fasciculate, embedded in persistent mycelial strands which join together in the neck of the perithecium, ovoid-oblong, gradually narrowed above, short stipitate, 8-spored, 49–81 × 8–10 μ (average 63 × 9 μ). Ascospores hyaline to greenish, fusiform-elliptic, uniseptate and constricted, the upper cell abruptly swollen just above the septum, the lower cell narrower and acute, guttulate, subdistichous, 12–16 × 4–6 μ (average 14 × 5 μ).

In corollas, leaves, and stems of cultivated *Chrysanthemum morifolium* Ram. collected in Pittsboro, North Carolina, June 1948.

Pycnidia abundant, mostly scattered on stems, but densely gregarious on petals, covered by the epidermis except where the short-necked ostiole protrudes, depressed-globose, 111–325 μ in diameter on stems (average 182 μ), somewhat smaller on petals, 72–180 μ (average 122 μ), texture parenchymatous, the cells rather large and thin-walled, amber, dark brown around the ostiole. Spores ovoid to cylindrical, straight or somewhat curved, mostly becoming septate, hyaline, faintly guttulate, usually without constriction until germination, 8–13 × 3–4 μ (average 10 × 3 μ), the non-septate conidia being 4–10 × 2–4 μ (average 6 × 3 μ).

In corollas, stems, and petioles of cultivated *C. morifolium* Ram. causing blight, Fayetteville and Raleigh, North Carolina. *Ascochyta chrysanthemi* F. L. Stevens, Bot. Gaz. **44**: 241–258. 1907.

Apparently Stevens did not distribute exsiccati of *Ascochyta chrysanthemi* on the host, but sent out bits of dried culture with pycnidia. Consequently we have distributed specimens of both *A. chrysanthemi* and *Mycosphaerella ligulicola* on *Chrysanthemum morifolium* to the following herbaria: Department of Plant Pathology, Cornell University, Ithaca, N. Y.; Department of Botany, University of California, Berkeley; Division of Plant Pathology, University of California, Los Angeles; Farlow Herbarium, Harvard University, Cambridge, Mass.; U. S. Department of Agriculture, Beltsville, Md.; Commonwealth Mycological Institute, Kew, England; Department of Plant Pathology, University of Georgia, Athens.

Cultures of the fungus have been placed in the Centraalbureau voor Schimmelcultures, Baarn, Holland, and in the American Type Culture Collection, Washington, D. C.

LIFE HISTORY AND ENVIRONMENTAL RELATIONS

This fungus maintains a high biotic potential through most of the year in the region of its principal destructiveness, and seems to have similar

potentialities in other areas having frequent rains during the chrysanthemum growing season.

Pycnidia form readily and abundantly in infected flowers, buds, and peduncles, and to a less extent in stems and leaves, under field and cloth-house conditions. During wet periods enormous numbers of conidia exude in gelatinous drops or short cirri and are splashed or blown about in raindrops, but are not detached and spread by air currents alone. With the usual crowding of plants under cultural conditions it is possible for a single infection to spread to many near-by flowers. It is probable that the gelatinous spores get on the black cloth covers which are pulled over the plants each day and that, in wet periods, they are thus spread along the beds. Workmen also may spread the spores on clothing, tools, or hands. Such situations are known for Septoria leaf spot of this crop (2).

Because only a few hours are required for successful infection of the petals, short periods of rain are sufficient to induce the disease. The wide temperature range favorable for infection and growth (6°–30° C.) indicates that moisture would generally be more of a limiting factor to development of an epidemic. Once established, the fungus apparently can survive drying very well and resume growth in the tissue when rewetted. If it is well established, growth continues even under dry conditions. The current practice of growing plants in cloth houses under black cloth to regulate day length and thus control flowering tends to retard drying of the petals following a rain, and may favor production of a new crop of pycnidia within a few days. The number and rate of production of pycnidia makes it possible for a given focus of infection to enlarge and produce extensive loss in a short time.

The fungus is well adapted in several ways to survive the period between chrysanthemum crops. The pycnidia in stems and flowers are able to survive the winter and the enclosed spores are viable in the spring. The overwintering of perithecia has already been discussed and the point established that mycelia may overwinter in stems and later give rise to perithecia.

Development of the disease in the first flowers of each new season may, on the basis of our field observations in North Carolina, start in several ways. A few mature perithecia were present at Pittsboro on old stems on June 8, 1948, and, judging from the numbers of perithecia produced on such stems shipped to Los Angeles, California, they continued to be formed until late fall. In fact, when the fields were visited in late October, 1948, there was ample evidence of recent ascospore infection of the flowers. It is probable that ascospore discharge and infection occurs through much of the flowering season in the field. While observational evidence of forcible discharge of asci is lacking, the "jack-in-the-pulpit" type of dehiscence (Fig. 3, I) is hardly one to release spores by any other method. The ascospores would be air-borne and would produce scattering

infections over the plants, as contrasted to the localized streaks of infection from water-borne conidia. It is not improbable, from the observed survival of the fungus, that it can overwinter as pycnidia on basal stems and leaves, and initiate infections in the spring by spores which splash upward, much as with Septoria leaf spot (2). Because of the infrequency of foliage or stem infections, this method of reaching the buds or flowers, often 3 feet above the ground, probably is not common.

The fungus commonly spreads into the peduncle from the infected corolla, and these bits of stem as well as other stem cankers are left in the field after harvesting. Ascospores produced on such stems may be carried by air currents to near-by fields of chrysanthemums.

That this fungus apparently did not spread out of the Southeastern States during a period of more than 40 years is difficult to explain. For the most part, propagative material has been shipped into but not out of this area, and spread thereby minimized. Until very recently almost no southeastern wholesale growers appear to have been shipping cut chrysanthemums into the Northern States. Recently, however, flowers have been extensively shipped, some of which must have been infected and hence dumped into rubbish piles. If such a pile was in the vicinity of flowering chrysanthemums, wind-borne ascospores might initiate infections and thus introduce the fungus to a new area. There apparently is nothing in the climate of the eastern, midwestern, and northwestern growing areas that would check the disease, once it was introduced. The dryness of the Southwestern States would seem to impose an effective limitation on the severity of the disease there if introduced.

The sequence of rainfall in the infested area in North Carolina may be important in the initiation of the perithecial stage. The mean monthly precipitation[9] of four stations in the area (Fayetteville, Raleigh, Durham, Greensboro) ranges from 3.41 to 3.80 in. from December through May, increases to 4.64–5.25 in. from June through August, then declines sharply to 3.63 in September, 2.72 in October, and 2.35 in November. The normal flowering months for chrysanthemum in that area are also the dry months in which perithecia would be most likely to mature. The development on the host under experimental conditions of immature perithecia in 13 days from actual infection indicates that their maturation and expulsion may occur within a relatively short time.

CONTROL

Cultural practices. Use of planting stock from areas in which the disease occurs should be avoided since it appears that the pathogen might well become established in any region characterized by frequent rainfall during the growing season. Likewise, rotted flowers from known infected areas should be burned, rather than dumped on refuse piles.

[9] Climate and Man. U. S. Dept. Agr., Yearbook 1941: 1035–1037. 1941.

Practices designed to minimize the carryover of inoculum from one growing season to the next may be of considerable value. Fields should be plowed deeply in the fall to bury completely all residual plant material. All stems, leaves, *etc.* from cut flowers should be burned or buried, never dumped into a near-by ravine or rubbish pile. Crop rotation for at least 2 years is advisable. Propagation should be entirely by tip cuttings from stock plants grown under glass or other rainproof cover, and irrigated so as to preclude splashing water.

The use of waterproof bags tied over the buds before they begin to open was tested by one grower. About 95 per cent of the flowers so protected were free of disease, whereas almost all noncovered flowers were lost. This method of control might be practicable in small plantings or exhibition stock. Overhead sprinkling also should be avoided.

Under garden conditions, wider spacing and improved ventilation of plants would expedite drying and be of some benefit in reducing losses. The removal of the first infected buds early in the season would delay the increase of inoculum.

Fungicides. Since complete elimination of ray blight by cultural manipulation appears improbable due to the potentialities of the sexual stage and possible carryover on undetermined weed hosts, the value of protective sprays was investigated.

A preliminary experiment tested zinc ethylene-bis-dithiocarbamate (Parzate) at 1 lb. per 100 gal., dichloronaphthoquinone (Phygon) at ½ and 1 lb. per 100 gal., phenyl mercury triethanol ammonium lactate (Puratized Agricultural Spray) at ½ and 1 pint per 100 gal., with Tergitol No. 7 at 1–800 as the spreader in each case. All were completely effective in preventing infection, but severe petal injury occurred with Phygon and Puratized, and slight injury with Parzate or with Tergitol alone. Checks were heavily infected by the fungus.

A second experiment used Parzate at ½ and 1 lb. per 100 gal. without spreader, and at 1 lb. per 100 gal. with Tergitol No. 7 at 1–2000, or DuPont IN 3622 at 1–1000 and 1–2000. Complete protection without petal injury was obtained in each case, while heavy infection developed on the checks. The deposit was much less conspicuous when a spreader was used.

In these tests the fungicides were atomized on the blossoms and foliage of susceptible chrysanthemums (Arcadia, Rev. Bushnell, Goldsmith) and allowed to dry before being atomized with a conidial suspension taken from field-infected flowers. Each lot of flowers was then maintained under a separate bell jar at laboratory temperatures for 5 days, when records were taken.

While it is possible that Phygon and Puratized would be safe and effective at lower concentrations and that other fungicides also would be satisfactory, Parzate was recommended tentatively to growers on the basis of these tests. Reports from a North Carolina grower indicated successful

control during the 1948 season with Parzate at ¾ lb. per 100 gal. plus 6 oz. Lux soap (for spreader) and various insecticides applied every 7 days up to the time of flowering. Sprays were applied in the bud and blossom stage using Parzate at ⅜ lb. per 100 gal. plus 4 oz. Dreft detergent. Effective control might be obtained with other zinc ethylene-bis-dithiocarbamates (*e.g.*, Dithane Z-78, Dithane D-14 + zinc sulfate) at equivalent concentration of active ingredient.

SUMMARY

Ascochyta ray blight of chrysanthemum has remained an important disease in North Carolina for more than 40 years without much spread to other areas. It occurs also in South Carolina, Mississippi, Florida, and Maryland. The fungus occurs in California. Sudden and severe commercial loss results from flower infection; stem and leaf invasion produces little injury.

Pycnidia are produced on petals, stems, or leaves under conditions of variable moisture and temperature. Conidia exude in gelatinous masses and are disseminated by water; at laboratory temperatures they germinate in 3½ to 4 hr. and infect petals in slightly more than 6 hr. Once the pathogen is established in the tissue it will continue growth and will cause the flower to decay under conditions of relative dryness. Infection and subsequent growth of the fungus in petals will occur at 6°–30° C., is highly favored at 20°–26° C., and is optimum at 24° C. Pycnidia form in petals within 13 days after infection. A very high biotic potential is mantained by the pathogen during the growing season.

Perithecia form quickly and profusely on infected stems, flowers, or leaves under moist, but also very dry conditions. They mature in late summer and fall, and appear to supply much of the primary inoculum for infection of developing buds and flowers in the fall and winter.

The previously unreported perfect stage of *Ascochyta chrysanthemi* Stev. is here described under the name, *Mycosphaerella ligulicola* sp. nov.

The fungus overwinters as mycelia or pycnidia, or as developing perithecia. Because of its wide tolerance of temperature and moisture it would seem to be a potentially dangerous parasite over a much wider area than its present range.

Various cultural practices designed to reduce inoculum carryover or to keep the budding or flowering chrysanthemum plants dry may aid in control. A Parzate spray (¾ lb. per 100 gal., plus a good spreader) applied on a 7-day schedule gave good control of the disease under field conditions.

UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA
AND
CORNELL UNIVERSITY
ITHACA, NEW YORK

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LOCAL SUSCEPTIBILITY OF COTYLEDONS AND LEAVES OF CUCUMBER TO TOBACCO MOSAIC VIRUS[1]

H. H. McKinney[2] and Robert W. Fulton[3]

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Cucumber (*Cucumis sativus* L.) has long been regarded as being immune from all types of infection by the virus of tobacco mosaic, but during the past four years many tests have shown that this plant and several related species are locally susceptible to this virus, to a known mutant of the virus, and to several viruses thought to be related to the tobacco mosaic virus.

MATERIALS

At Beltsville, Maryland, the studies were conducted with the following seven virus isolates:

(1) Tobacco mosaic virus obtained from James Johnson in 1924.

(2) Mutant *BSY* (6), previously designated Type A yellow mosaic virus (4), which was isolated from the tobacco mosaic virus listed above.

(3) Tobacco mosaic virus obtained from H. A. Allard.

(4) Aucuba yellow mosaic virus of tobacco from John Caldwell, Essex, England.

(5) A light green mosaic virus which was associated with the above aucuba yellow mosaic virus.

(6) A virus isolated from *Plantago major* L. (7). This virus induces symptoms in some individuals of *Plantago Rugelii* Dene., and it is very similar to, if not identical with, virus isolated from a few plants of *P. Rugelii* found near pathways and roadsides on the Station grounds at Beltsville, Maryland, and in Arlington, Virginia.

(7) Mild dark-green mosaic virus from *Nicotiana glauca* R. Grah. collected in the Canary Islands (3).

The tobacco mosaic virus from James Johnson has been used continuously by one of the authors (McKinney) since 1924, and it has been regarded as the type virus of tobacco mosaic in all of this author's studies. The mosaic mottling induced by this virus in tobacco has been illustrated in several publications (4, Fig. 1, B; 5, frontispiece B). The tobacco mosaic virus from H. A. Allard had been stored by him for 15 years previous to 1931, when a portion of it was used in the mutation studies (4). From 1931 to 1949 the

[1] This paper combines two sets of observations and data collected independently.

[2] Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

[3] Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture; and the Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

portion of the virus used in the present studies had been stored in air-dried tobacco leaf tissue at room temperature.

Beans (*Phaseolus vulgaris* L.) of the variety Scotia, *Datura stramonium* L., and the hybrid *Nicotiana tabacum* × *N. glutinosa* were used for assaying virus in the cucurbit tissues.

At Madison, Wisconsin, the studies were conducted with 8 strains of tobacco mosaic virus obtained from James Johnson (2), with the masked strain of tobacco mosaic virus isolated by Holmes (1), and with an undescribed virus isolate.

The undescribed virus was isolated from field-grown tobacco plants that had been severely stunted and had numerous small necrotic spots on slightly mottled leaves. When inoculated to the hybrid test plant, local lesions typical of tobacco mosaic virus were produced. The thermal inactivation point and resistance to drying were typical of tobacco mosaic virus, and in serological tests the virus was precipitated by antiserum to common tobacco-mosaic virus. The virus produced local lesions on healthy leaves of *Nicotiana sylvestris* Speg. and Comes, but no lesions were produced on leaves of this plant which had been inoculated 3 days before with common tobacco-mosaic virus. It was concluded that the new isolate is a strain of tobacco-mosaic virus. For convenience it is designated here as the KC strain.

METHODS

Inoculations were made by the wiping method, with and without the use of 600-mesh carborundum dust. In most of the tests the cucurbit cotyledons or leaves were inoculated with fresh virus extracts obtained from the leaves of growing tobacco plants. However, in some of the tests conducted at Beltsville the inoculations were made with virus that had been stored for long periods in air-dry leaf tissue at room temperatures. All plants were grown in small clay pots in greenhouses, or in culture chambers. At Beltsville, two methods of temperature management were followed: (1) 34° C. following inoculation and through the first night, followed by greenhouse temperature as near to 24° C. as possible to the end of the test; (2) as near 24° C. as possible, throughout the test. In bright warm weather the greenhouse temperatures were kept as low as possible by shade and ventilation. At Madison most of the cucumber tests were conducted in a greenhouse with the temperature controlled near 27° C., but some tests were conducted at higher temperatures in a culture chamber.

In the tests at Beltsville the multiplication of virus in the cucurbit cotyledons and leaves was determined by direct assay of these tissues at suitable times after inoculation. Tissues were diluted 3 to 5 times in distilled water. Some virus will remain on a wiped surface, even when rinsed under the stream of water from a faucet. To reduce error from this source, the inoculated cotyledons and leaves were soaked for 15 minutes in a M/0.1 solution of trisodium orthophosphate at room temperature. In some tests a

M/0.2 solution was used. The tissues were then rinsed thoroughly in virus-free water before being pulped and extracted for assay. Tests with virus-impregnated soft filter paper which was thoroughly dried and then soaked in solutions of trisodium orthophosphate leave no doubt as to the effectiveness of this chemical for killing residual virus on inoculated cotyledons and leaves. Never have more than the slightest traces of virus survived in the filter paper tests, and usually none survives.

In making the assays the lesion counts were calculated on a unit area basis. The areas of the wiped leaves of bean seedlings and Datura seedlings were determined within rather close limits by matching with paper patterns, the areas of which had been predetermined.

In the studies at Madison the multiplication of virus in the cucurbit cotyledons and leaves was determined by serial transfer inoculations from cucumber to cucumber, and by direct assay of the inoculated cotyledons on the hybrid test plant (*Nicotiana tabacum* × *N. glutinosa*). The lesion counts on this hybrid are given in terms of the total or average per leaf. It should be kept in mind that the area of one of these leaves is much greater than the area of a leaf of a bean seedling or of *Datura stramonium*.

RESULTS OF TESTS WITH CUCUMBER AND RELATED SPECIES

Symptoms Induced on Cucumber

Local lesions usually appeared within 4 to 7 days when cucumber cotyledons were inoculated with tobacco mosaic virus. Fresh virus from young tobacco plants, diluted 3 to 5 times in water, has induced 50 to 100 local lesions per cotyledon on Early White Spine cucumber. The lesions on cucumber are illustrated in figure 1. The yellow mosaic virus mutant *BSY*, the mild dark-green mosaic virus, aucuba mosaic virus, and the KC strain induced especially distinct lesions on cucumber cotyledons. The virus from *Plantago major* induced less distinct lesions. In several of the tests at both Beltsville and Madison lesions failed to appear on part or all of the cotyledons that had been inoculated with some of the viruses used. Assays showed that when the inoculum contained a sufficient amount of virus there was an appreciable amount of virus in these lesion-free cotyledons.

At Beltsville the cucumber varieties Early White Spine, National Pickling, Boston Pickling, Improved Long Green, and Early Cluster have shown nearly the same reaction characteristics. At Madison the KC strain produced similar symptoms on all of 17 varieties of cucumber.

Increase of Virus in Cucumber

The extent to which virus increase occurs in cucumber was demonstrated with dilutions of 10, 100, 1000, and 10,000 times in distilled water of an extract from cucumber cotyledons that had been inoculated 16 days previously with the Johnson virus. Any residual virus on the cotyledons was inactivated before processing. Each dilution sample was wiped on the pri-

mary leaves of 10 bean seedlings. Local lesion counts per cm² of leaf were 14.0, 4.5, 0.6, and 0.09, respectively, for the above four dilutions.

The Johnson virus heated for 10 min. at 87° C. infected one tobacco plant out of the 5 plants inoculated. Virus from this plant was used to inoculate the cotyledons and first leaf of 10 Early White Spine cucumber seedlings. The small chlorotic lesions that appeared were more numerous on the cotyledons than on the leaves. When the cotyledons were assayed on the leaves of *Datura stramonium*, local lesions were produced at the rate of 10 per cm.² of leaf. Assay data on seven virus isolates are shown in table 1. Variations in the lesion counts shown in the table are attributed chiefly to differences in the activity of the virus used to inoculate the cucumber cotyledons. The two lowest counts shown in the table are from virus that had been stored in dry tissue for long periods at room temperature, and used directly to inoculate the cotyledons.

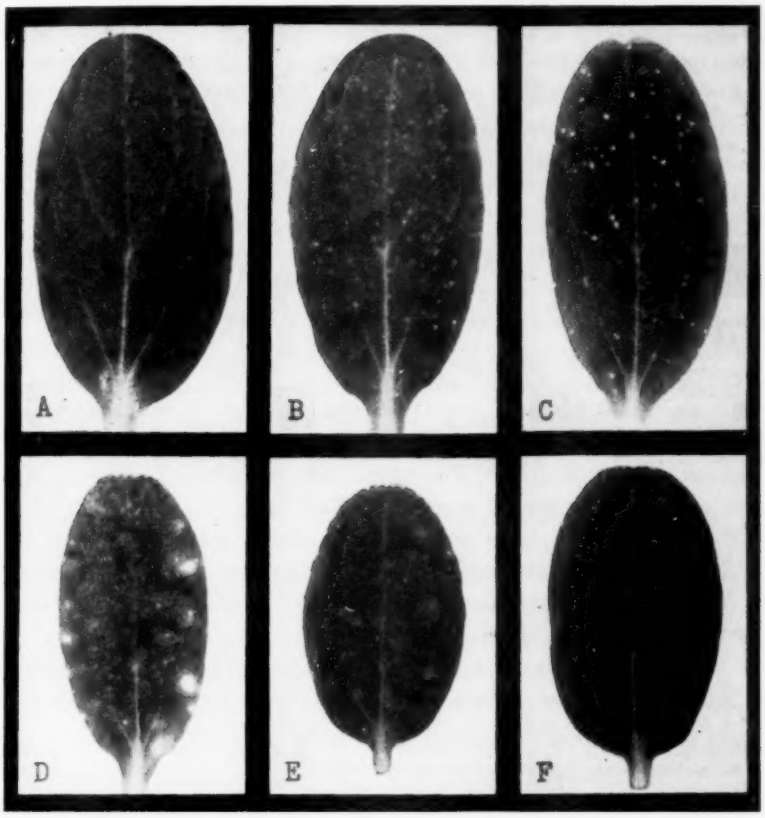

FIG. 1. Local lesions caused by strains of tobacco mosaic virus on the cotyledons of Boston Pickling (upper row) and Early White Spine (lower row) cucumber. A. Healthy control wiped with fresh juice from a healthy tobacco plant. B and D. Johnson's virus. C. KC virus. E. *BSY* virus. F. Virus from *Plantago major*.

TABLE 1.—*Assay data indicating an increase of virus in the cotyledons of cucumber inoculated with several collections of tobacco mosaic virus. Tissues were diluted 3 to 5 times in distilled water. Datura stramonium L. was used for the assays*

Virus used	Variety of cucumber inoculated	Virus in cucumber cotyledons (No. of lesions per cm² of test leaf)
Tobacco mosaic (James Johnson)	Early White Spine	30.0
	Boston Pickling	7.4
Tobacco yellow mosaic (Yellow mosaic mutant *BSY*)	Early White Spine	40.0
	Early Cluster	2.6
Tobacco mosaic (H. A. Allard)	Early White Spine	30.0
Tobacco aucuba (yellow) mosaic	do	10.0
Tobacco mosaic associated with aucuba mosaic	do	11.0
Mild dark-green mosaic from *Nicotiana glauca*, Canary Islands	do	30.0
Isolate from *Plantago major*	do	40.0

Data obtained at Madison, and presented in table 2, showed that several of the isolates of tobacco mosaic virus can be maintained through 5 to 10 serial passages on cucumber. The cucumber cotyledons from each transfer were assayed on the hybrid test plant. With some of the viruses (KC, isolates 1, 2, and 5, and Holmes' masked), cucumber cotyledons from the last transfer produced as many lesions as from the first. Other isolates could not be maintained on cucumber, perhaps partly because the original inoculum did not contain sufficient virus.

New leaves and stems of cucumber plants that had been inoculated previously by wiping the cotyledons and first leaf with the several viruses under

TABLE 2.—*Results of serial transfers at 6- to 8-day intervals of 10 isolates of tobacco mosaic virus in the cotyledons of cucumber*

Strain of virus used	Number of transfers from cucumber to cucumber	Average number of local lesions per hybrid test leaf inoculated with cucumber cotyledons from each transfer
KC	10[a]	83
1	6[a]	131
2	6[a]	114
3	3[b]	4
4	2	29
5	5[a]	41
6	4	54
7	1[b]	5
8	2	16
Holmes' masked	6[a]	40

[a] No attempts were made to carry these transfers further.
[b] Two additional attempts failed to maintain each of these viruses on cucumber.

study were assayed on many occasions. In no case was it possible to detect virus in these tissues, which indicated that the virus had not moved in from the inoculated sites. At Beltsville, one plant of cucumber, variety National Pickling, was allowed to grow for about 1 month after inoculation with the Johnson virus through the cotyledons and first leaf. At the end of this period the right half of each leaf from the second to the seventh inclusive was inoculated with the Johnson virus. A few small chlorotic lesions appeared on the leaves. Each inoculated half-leaf was assayed on the primary leaves of 5 bean seedlings. Local necrotic lesions were produced at the rate of 3.75, 0.65, 0.50, 1.67, 2.32, and 0.55 per cm^2, on test leaves 2 to 7, inclusive. The cucumber half-leaves that were not inoculated were bulked and assayed on the primary leaves of 5 bean seedlings. No local lesions or other signs of infection appeared.

In trials at Madison, cucumbers were inoculated with several of the virus isolates under study, and the plants were cultured at 35° C. and at 38° C. for 8 to 10 days. After an additional 14 days at 27° C. the leaves and cotyledons of the plants were assayed separately. No virus could be demonstrated in the noninoculated leaves, while the inoculated cotyledons produced from 30 to 100 lesions per leaf on the hybrid test plant.

Tests on Other Cucurbits

The cotyledons of watermelon (*Citrullus vulgaris* Schrad., var. Dixie Queen), squash (*Cucurbita pepo* L. var. White Scallop Bush), and gourds (*Cucurbita pepo*, mixed) were inoculated with a very active fresh extract of the Johnson virus. The lesions on watermelon were well-defined necrotic spots, a few of which penetrated to the underside of the cotyledons. The lesions on squash and gourds were faint gray specks that were very slow to appear. Assays on *Datura stramonium* after 10 days gave 2.4, 7.0, and 7.5 lesions per cm^2 of leaf, respectively, for the cotyledons of watermelon, squash, and gourd. In spite of the well-defined lesions on the cotyledons of watermelon, the assays have always shown low virus activity in this species.

The KC strain of virus was inoculated to cotyledons of watermelon (var. Stone Mountain), squash (var. Butternut), pumpkin (*Cucurbita pepo* L. var. Connecticut Field), and muskmelon (*Cucumis melo* L. var. Bender's Surprise). The lesions on watermelon were small but distinct orange to brown spots. On the other species the lesions were indistinct. Assays from inoculated cotyledons gave from 30 to 80 lesions per leaf of the hybrid test plant, except in the case of watermelon, from which virus could not be recovered.

CONCLUSIONS

It has been known for many years that the tobacco mosaic virus does not infect cucumber systemically, and apparently it had been assumed that this host was also immune from local infection. However, it appears from the results presented that the capacity for infecting cucumber locally is a rather

general property of this virus and its strains. Failure to observe this phenomenon in the past can be explained by the fact that the local lesions are not always striking on cucumber cotyledons, especially when the concentration of virus in the inoculum is not reasonably high.

It is of interest that the mild dark-green mosaic virus, which is regarded as being more distantly related to the tobacco mosaic virus than an ordinary mutant, also induces local infection in cucumber.

It appears that cucumber and certain other cucurbits have greater value than has been supposed for the identification of tobacco mosaic virus and some of its mutants and more distant relatives.

U. S. DEPARTMENT OF AGRICULTURE
AND
WISCONSIN AGRICULTURAL EXPERIMENT STATION
MADISON, WISCONSIN

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INCIDENCE OF LIMA BEAN ROOT ROT IN SOILS TREATED WITH FUMIGANTS AND INSECTICIDES FOR CONTROL OF WIREWORMS[1]

JOHN T. MIDDLETON, M. W. STONE, AND J. B. KENDRICK, JR.[2]

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INTRODUCTION

Lima beans (*Phaseolus lunatus* L.) grown in the southern coastal counties of California are subject to root rot caused by *Fusarium solani* f. *phaseoli* (Burk.) Snyder and Hansen, *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn, and *Thielaviopsis basicola* (Berk.) Ferraris, individually or collectively (13). The results of a several years' survey of areas producing Lima bean seed seemed to indicate that root rot is generally more prevalent in light soils than in heavy soils, and further, that these light soils usually contain relatively large populations of nematodes (*Heterodera marioni* (Cornu) Goodey) and of the sugar-beet wireworm (*Limonius californicus* Mann.). This suggested a possible relationship between soil fauna and root rot of Lima beans. With the advent of measures devised to control both nematodes and wireworms, an opportunity was afforded to check this hypothesis.

The Ventura County survey to determine the incidence of Lima bean root rot was conducted in field plots located on the Borchard and Bruns ranches near Oxnard, California. These plots were also used for testing the efficacy of various soil fumigants and insecticides for the control of the sugar-beet wireworm, and for two years (1946–1947) data were collected for the purpose of testing the validity of the contention that the soil fauna preying on Lima bean roots predisposes the host to infection by the several root-rotting organisms. Results are reported in the present paper.

MATERIALS AND METHODS

A dichloropropane-dichloropropylene mixture and an ethylene dibromide solution were tested as soil fumigants, and technical benzene hexachloride and technical dichlorodiphenyltrichloroethane (DDT) were tested as insecticides. The dichloropropane-dichloropropylene mixture contained approximately 33 per cent 1,2 dichloropropane, 63 per cent 1,3 dichloropropylene, and 4 per cent heavy trichlorides of propane. The ethylene dibromide solution contained 10 per cent ethylene dibromide by volume in a naphtha 200-base thinner. The technical benzene hexachloride con-

[1] Paper No. 597, University of California Citrus Experiment Station, Riverside, California.

[2] Respectively, Associate Plant Pathologist, University of California, Riverside; Entomologist, U. S. Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Ventura, California; and Junior Plant Pathologist, University of California, Riverside.

tained 12 per cent gamma isomer. The DDT and benzene hexachloride were diluted with pyrophyllite before application.

In each experiment the treatment and control were replicated four times in randomized blocks. Lima beans of the Ventura variety were planted in each field between May 3 and 10, at the rate of 90 lb. per acre. The wireworm population of the bean rows was determined by taking eight soil samples 4 in. wide, 4 in. deep, and 3 ft. long, at random, in the center 10 rows of each plot, between the fifth and seventh days after planting. Each sample was sifted through a 12-mesh screen to recover the wireworms present.

Soil fumigants were tested in ¼-acre plots of Yolo fine sandy loam soil in Borchard field A (Table 1). The dichloropropane-dichloropropylene mixture was applied at the rate of 18, 26, and 36 gal. per acre, and the ethylene dibromide solution at the rate of 22 gal. per acre. These materials were applied 6 to 7 in. deep, at 12-in. spacings, by means of a small, continuous-flow-type soil-fumigant applicator. At the time of treatment (April 10, 1946), the moisture content of the soil ranged from 6.4 per cent, by weight, at the 4-in. depth to 13.1 per cent at the 12-in. depth; soil temperatures ranged from 64° to 67° F. at the 6-in. depth and from 63° to 66° at the 12-in. depth.

The same soil fumigants were also tested in ⅓-acre plots of Yolo very fine sandy loam in Bruns field A (Table 1). The dichloropropane-dichloropropylene mixture was applied at the rate of 22 gal. per acre, and the ethylene dibromide solution at the rate of 21 gal. per acre. These materials were applied 7 to 8 in. deep, at 12-in. spacings, by means of a commercial soil-fumigant applicator. At the time of treatment (April 8, 1947), the moisture content of the soil averaged 7.3, 9.1, 10.7, and 12 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; soil temperatures ranged from 56° to 68° F. at the 4-in. depth and from 60° to 63° at the 12-in. depth.

Insecticides used in Borchard field B (Table 1) were applied as dusts on the soil surface of plots $\frac{2}{5}$-acre in size. The materials, benzene hexachloride (4 lb. per acre) and DDT (20 lb. per acre), were incorporated with the soil by disking and cross-disking to a depth of 8 in. and then roll-harrowing. At the time of treatment (April 23, 1946), this Yolo fine sandy loam soil had a moisture content of 11.1, 14.4, 16.2, and 18.1 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; and soil temperatures ranged from 63° to 65° F. at the 6-in. depth.

These same materials were also tested in Bruns field B (Table 1) in a randomized block experiment, with four replicated plots ½-acre in size for each treatment. The benzene hexachloride was applied at the rate of 4 lb. per acre, and the DDT at 10 lb. per acre. The test area had been planted to a covercrop of sweet clover, and the dust treatments were applied on the covercrop before it was plowed under. At the time of treatment (March 20, 1947), the Yolo very fine sandy loam soil in these plots was

rather wet, owing to a previous irrigation. Soil moisture averaged 11.5, 13.0, 13.4, and 13.5 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; and soil temperatures averaged 70°, 66°, and 65° F. at the 4-, 8-, and 12-in. depths, respectively.

Samples consisting of 10 bean plants from each of four replicate plots were collected about 6 weeks after seeding. A root-rot injury rating of the several samples was made, utilizing five arbitrary classes from 0 to 4, which represent approximately 0, 25, 50, 75, and 100 per cent injury of the hypocotyl surface. This rating was transformed into an injury index in which the index is the sum of the products of each class value and the number of individuals within that class, divided by the total number of individuals in the sample. A portion of each rated hypocotyl was removed and plated on cornmeal agar. The cornmeal agar plates were examined after sufficient time had elapsed to permit all fungus growth to become evident, and the total number of fungus colonies was then recorded.

RESULTS

It may be observed from the data presented (Table 1) that the effect of all treatments was to reduce the value of the injury index.

The fungi isolated (Table 1) comprised principally those species concerned with the root-rot complex, but there were also occasional isolates of *Fusarium oxysporum* Schl., *F. roseum* (Lk.) Snyder and Hansen, *Rhizopus* sp., and *Trichoderma lignorum* (Tode) Harz.

Wireworm control (Table 1) was effected by all treatments, with greatest population decrease with soil applications of ethylene dibromide solution. No data were secured for nematode populations because of the lack of a suitable technique permitting population estimates upon immature plant samples.

There is a direct relationship between the injury index and the number of fungi isolated from the bean plants sampled in all plots. This relationship is more conclusively illustrated by the ratio of the injury index to the number of fungi isolated (Table 1), and notable uniformity of correlation may be noted within the three areas sampled.

There is likewise a direct relationship between the number of wireworms per foot of bean row and the injury index, as illustrated by the calculated ratios (Table 1). A comparison of the two classes of ratios given reveals immediately that there is a proportionately greater reduction in wireworm population with treatment than there is corresponding decrease in injury index.

EFFICACY OF MATERIALS AS FUNGICIDES

Dichloropropane-dichloropropylene mixture and ethylene dibromide solution have only recently been used as soil fumigants; this limits the accumulation of much pertinent literature.

TABLE 1.—*The incidence of Lima bean root rot in field plots treated for the control of the sugar-beet wireworm*

Treatment, plot location, and material used	Amount per acre	Root-rot injury index	Number of fungi isolated	Number of wireworms per foot of bean row[a]	Ratio of	
					Injury index to number of fungi	Number of wireworms to injury index
Soil fumigant						
Borchard field A:						
Dichloropropane-dichloropropylene mixture	18 gal.	2.7	49	0.3	0.06	0.11
do	26 gal.	2.2[b]	36	0.2	0.06	0.09
do	36 gal.	1.8[b]	28	0.1	0.06	0.06
Ethylene dibromide solution	22 gal.	2.8	43	0.0	0.06	0.00
Control (no treatment)		3.6	63	5.5	0.06	1.53
Bruns field A:						
Dichloropropane-dichloropropylene mixture	22 gal.	1.6	87	0.18	0.02	0.11
Ethylene dibromide solution	21 gal.	1.7	63	0.02	0.03	0.01
Control (no treatment)		2.3	89	1.03	0.03	0.45
Insecticidal dust						
Borchard field B:						
Benzene hexachloride	4 lb.	2.4	56	0.2	0.04	0.08
DDT	20 lb.	2.5	47	0.7	0.05	0.28
Control (no treatment)		2.7	67	3.1	0.04	1.15
Bruns field B:						
Benzene hexachloride	4 lb.	1.4	64	0.2	0.02	0.14
DDT	10 lb.	1.4	71	0.3	0.02	0.21
Control (no treatment)		2.2	87	3.8	0.02	1.73

[a] A difference of 1.1, 0.8, 0.2, and 0.8 wireworms per foot of soil (4 × 4 × 12 in.) required for significance at 5 per cent level in Borchard fields A and B and Bruns fields A and B, respectively.

[b] Significantly different from the control at the 5 per cent level, according to the "t" test for group comparison.

Carter (2) first reported the use of dichloropropane-dichloropropylene mixture as a nematocide in 1943. Application of 15 gal. (150 lb.) of this mixture per acre corrected pineapple plant failure, which Carter states is induced by "at least *Anomala* beetle larvae (*A. orientalis*), nematodes and pythiaceous fungi." The pythiaceous fungi presumably include *Phytophthora cinnamomi* Rands, *P. parasitica* Dast., and *Pythium* spp. (9).

Parris (12) reports dichloropropane-dichloropropylene mixture to be an efficient nematocide but a poor fungicide, at dosages of 100 gal. per acre, for the control of damping-off of pea and spinach in soil infested with *Rhizoctonia* cf. *solani* Kühn and *Fusarium* cf. *martii* A. and W., and tomato wilt caused by *F. oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. Dosages of 55 gal. per acre did not reduce post-emergence loss of beans caused by *Pythium aphanidermatum* (Edson) Fitzpatrick. Newhall (10) confirms the finding that dichloropropane-dichloropropylene mixture is effective as a nematocide, and adds that "it has rather limited fungicidal and herbicidal properties."

Stark and Lear (14) report favorably upon the nematocidal properties of both dichloropropane-dichloropropylene mixture and ethylene dibromide solution, and add that dichloropropane-dichloropropylene mixture has fungicidal qualities at dosages higher than required for nematode control, but that ethylene dibromide solution was a relatively poor fungicide. The addition of 0.50 ml. of dichloropropane-dichloropropylene mixture to a 1-gal. glazed crock containing soil infested with *Fusarium* sp. permitted significantly greater emergence of pea seedlings than untreated soil. If we may assume that the 0.50-ml. dosage is received by a gallon of soil, such a dosage may be converted to gallons per acre by calculating the number of gallons of soil in an acre-foot and relating the charge applied. The milliliter dosages given by Stark and Lear (14) may then be multiplied by the factor 85.5 and expressed as gallons per acre. A 0.50-ml. dosage is therefore equivalent to 42.7 gal. per acre. This dosage of ethylene dibromide solution caused injury to pea seeds sown 8 to 14 days after treatment. A dosage of 0.25 ml., or 21.4 gal. per acre, did not cause pea-seed injury and failed to reduce significantly the damping-off of peas.

Zentmyer and Klotz (18) report that *Phytophthora cinnamomi* and *P. citrophthora* (Sm. and Sm.) Leonian are killed in soil by a mixture of 50 per cent 1,2 dichloropropane and 50 per cent 1,3 dichloropropylene, at a dosage of 0.15 ml. per qt. of soil, or about 51 gal. per acre; and by ethylene dibromide solution at a dosage of 0.27 ml. per qt. of soil, or approximately 92 gal. per acre. Using the above components separately, they determined that 1,2 dichloropropane was nontoxic to the fungi in soil at dosages of about 20, 51, and 92 gal. per acre; and that 1,3 dichloropropylene was nontoxic at about 20 gal. per acre, but was toxic at dosages of approximately 51 and 92 gal. per acre.

McClellan, Christie, and Horn (8) recently reported that under the con-

ditions of their experiments dichloropropane-dichloropropylene mixture was relatively ineffective, and ethylene dibromide solution had no effect, against *Fusarium oxysporum* f. *callistephi* (Beach) Snyder and Hansen, but that ethylene dibromide did reduce the viability of sclerotia of *Sclerotium rolfsii* Sacc. The fumigants were applied at the rate of 2.5 ml. to 46.5 lb. of soil in containers having a calculated capacity of about 7 gal. The description of methods does not permit ready determination of dosage, but the present writers estimate the 2.5-ml. charge to be equivalent to 23 to 30 gal. per acre.

Despite the large volume of literature published on DDT, there is a paucity of information concerning its value as a fungicide. Granovsky (3, 4) reported that 5 per cent DDT dust formulations had considerable fungicidal value for the control of late blight of potatoes, caused by *Phytophthora infestans* (Mont.) de Bary. Norris (11) published data demonstrating conclusively that 1 per cent DDT in culture media was nonfungicidal to *Ascochyta imperfecta* Pk., *Colletotrichum trifolii* Bain, *Ophiobolus graminis* Sacc., *Pleospora herbarum* (Pers.) Rab., and *Pseudoplea trifolii* (Rostr.) Petr. Norris also demonstrated that pea seed dusted with 50 per cent DDT and planted in infested soil gave 0 to 1 per cent emergence, whereas pea seed dusted with Spergon (tetrachloroparabenzoquinone) and sown in the same soil gave 86.5 per cent emergence. He further reports that undiluted DDT did not prevent development of wheat flag smut, caused by *Urocystis tritici* Körn. Heuberger and Wolfenbarger (6) ascertained that 0.75 lb. DDT per 100 gal. of water, alone and in combination with copper oxychloride or zinc dimethyldithiocarbamate, had no effect upon the control of early blight of potato, caused by *Alternaria solani* (E. and M.) Jones and Grout. Abbott (1), working with the slime mold *Physarella oblonga* Morgan, showed that there was no reduction in growth when the amount of DDT added to the medium was varied from 1.6 to 105.0 mg. per ml. Surface applications of DDT at the rate of 40 mg. per sq. cm. retarded the growth rate but not the ultimate amount of growth; reduction of growth rate was attributed to physical change of the surface of the medium. Horsfall (7) indicates that DDT is not fungicidal in conjunction with studies directed toward developing new fungicides. Timonin (15) demonstrated that 5 to 20 mg. of DDT per 100 gm. of culture medium was nontoxic to *Saccharomyces ellipsoideus* R., but that alcohol production was decreased and the rate of fermentation delayed for the initial 5 days. Similar results were secured with grapes sprayed with DDT. Wilson and Choudhri (16) report that DDT had no effect upon the growth of pure cultures of *Rhizobium* spp. from sundry leguminous hosts, and did not inhibit growth of *Aspergillus niger* v. Tiegh., *A. oryzae* (Ahl.) Cohn, *Penicillium camemberti* Thom, *P. expansum* Lk., *P. italicum* Weh., *P. roqueforti* Thom, *Chlorella* sp., 6 unidentified species of fungi, 10 species of bacteria, 5 species of algae, and 3 species of Actinomycetes. Heuberger and Stearns (5) state that "DDT has little or no fungicidal value."

Wilson and Choudhri (17) report that the application of 20 per cent crude benzene hexachloride to a number of heterotrophic soil organisms did not affect their ultimate growth, but that the growth of three species of algae was inhibited. The growth of pure cultures of *Rhizoctonia solani* was inhibited by the presence of pure delta isomer; no information was given for the effect of gamma isomer on this fungus.

DISCUSSION

The data (Table 1) show that there is a direct relationship between treatments and the amount of Lima bean root rot; they also show that mature wireworm populations were reduced by the treatments, but that the rate of reduction of wireworm populations was greater than the rate of reduction of disease incidence. This suggests not only that there is a relationship between wireworm populations and the incidence of Lima bean root rot, but also that treatment directly affects the incidence of the disease. Since there is a more rapid reduction of wireworms than of injury indices, it is assumed that wireworms play only a minor role in predisposing Lima beans to root rot. This is illustrated by the data, which show that the injury indices decrease with increases in dosages of dichloropropane-dichloropropylene mixture, whereas the wireworm populations are not so effectively reduced by applications of this mixture as by applications of ethylene dibromide solution; the fungicidal efficacy of dichloropropane-dichloropropylene mixture is thus demonstrated.

Treatment with ethylene dibromide solution reduced the injury index below that of the untreated plot. This reduction may have been due almost entirely to the elimination of the predisposing action of wireworm populations rather than to any fungicidal activity of the material. Similarly, benzene hexachloride and DDT treatments reduced the injury index below that of untreated areas, although less than did dichloropropane-dichloropropylene mixture; this again suggests that the reduction of injury indices was due more to the elimination of the predisposing effect of wireworms than to any fungicidal action.

Except for the reports of Granovsky (3, 4) and Parris (12), the preceding review of the relative fungicidal efficacy of the materials used would apparently support the contention that only the dichloropropane-dichloropropylene mixture has fungicidal properties. Granovsky fails to present sufficient data to permit ready interpretation of his conclusion, and until additional information is supplied the writers feel obliged to consider DDT as having little or no fungicidal value. Parris demonstrates conclusively that, at dosages employed, dichloropropane-dichloropropylene mixture was not an effective fungus eradicant, although at maximum dosages there was apparently a decrease in fungus populations. Since the results and interpretations presented here are concerned primarily with changes in numbers of fungi, and not their eradication, the findings of Parris are not alien to the problem discussed.

Root rot of Lima beans may operate independently of wireworm populations, but it is evident that there is a relationship between the soil fauna and the incidence of root rot, as hypothesized, and that wireworms may predispose the beans to root rot. Although no data were secured on nematode populations, it is interesting to note that applications of ethylene dibromide solution, which is reportedly an efficient nematocide, did not substantially reduce the amount of root rot in any trial, despite the virtual elimination of wireworm populations. This suggests that nematodes play little if any part in predisposing Lima beans to root rot.

SUMMARY

Root rot of Lima beans (*Phaseolus lunatus* L.) is a complex involving the fungi *Fusarium solani* f. *phaseoli* (Burk.) Snyder and Hansen, *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn, and *Thielaviopsis basicola* (Berk.) Ferraris.

The injury index is directly related to the number of fungi present on the subterranean portion of infected Lima bean plants.

Applications of the fumigants dichloropropane-dichloropropylene mixture and ethylene dibromide solution, and of the insecticides benzene hexachloride and DDT, to soil supporting Lima beans reduced the injury index over that for Lima beans grown on untreated soil.

A relationship exists between the number of wireworms and the injury index, although this relationship is not so pronounced as that between the injury index and the number of fungi. In general, the number of wireworms decreases more rapidly with treatment than does the injury index.

Reduction in disease incidence seems to be associated more closely with liquid applications, especially with dichloropropane-dichloropropylene mixture, than with dust applications. This suggests a fungicidal effect due to dichloropropane-dichloropropylene mixture.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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THE ACTION OF ANTIBACTERIAL SUBSTANCES ON THE GROWTH OF PHYTOMONAS TUMEFACIENS AND OF CROWN GALL TUMOR TISSUES[1]

R. S. DE ROPP

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Action of a crude preparation of penicillin on the crown gall organism and on galls produced by this organism was described by Brown and Boyle (1), who found that the crude metabolic liquid from a culture of *Penicillium notatum* inhibited the growth of *Phytomonas tumefaciens in vitro.* When introduced into crown galls on *Bryophyllum* by means of needle puncture, it caused the recession of these galls. Subsequently, Brown (2) reported that both penicillin and streptomycin were capable of destroying gall cells without injuring normal cells, the antibiotic substances causing destruction of the nuclei of the gall cells. "Cures of crown gall" were also reported by Hampton (3) in various plants which had been treated with penicillin and streptomycin, both the commercial and locally produced crude preparations.

Evaluation of this work is made difficult by the fact that impure preparations of these antibiotic substances appear to have been used. Crude preparations of these substances contain many compounds which may exert some action on plant growth. Also the findings of Brown and Boyle regarding the sensitivity of *Ph. tumefaciens* to penicillin appear to conflict with those of Waksman *et al.* (6), who state that, compared with Gram positive bacteria, *Ph. tumefaciens* must be considered as "fairly resistant" to penicillin. Experiments were therefore undertaken to explore this subject further, using the purest available preparations of antibiotic substances and employing tissue culture methods for growing both normal and tumor tissue.

MATERIALS AND METHODS

Three categories of experiments were performed. First, different strains of *Ph. tumefaciens* were treated in broth culture with different concentrations of antibacterial substances to determine what concentration of these substances would inhibit bacterial growth. Second, bacteria-free crown gall tissues of *Helianthus annuus* and *Vinca rosea* were cultured on a nutrient medium containing antibiotic substances and their growth compared with that made on nutrient agar lacking these substances. Normal tissues from these plants were similarly tested. Third, segments of sterile carrot tissue were inoculated with a virulent strain of *Ph. tumefaciens* and subsequently treated with antibiotic substances to determine whether these compounds had the capacity to inhibit tumor formation.

[1] This work was done in part under an American Cancer Society grant recommended by the National Research Council Committee on Growth. The author is indebted to Miss E. Pieczur for technical assistance.

The antibacterial substances used in this work were obtained from the following sources: streptomycin calcium chloride complex from Commercial Solvents Corporation; crystalline potassium penicillin G and streptomycin from R. T. Major of Merck and Co.; chloromycetin from Parke, Davis and Co.; aspergillic acid from G. Rake of the Squibb Institute for Medical Research; patulin from H. Raistrick; tolu-p-quinone, recrystallized material from Eastman Kodak Co.; A 377 hydrochloride (aureomycin) from Lederle Laboratories.

The strains of tumor tissue used were K1 from an induced tumor on sunflower and V 60 isolated by White (7) from *Vinca rosea*. The mode of obtaining normal tissue of *Vinca rosea* and *Helianthus annuus* has been described previously (4). The carrot tissue was removed from washed carrots by means of a sterile cork borer of 1 cm. diameter. Cylinders of tissue thus removed were cut into 6-mm. lengths and cultured on sucrose agar as employed for the culture of tumor tissues. Suspensions of *Ph. tumefaciens* having approximately known viable counts were applied to the cut surfaces of these tissue fragments, which were subsequently treated with the antibiotic substances to be tested.

THE ACTION OF ANTIBACTERIAL SUBSTANCES ON THE GROWTH OF PHYTOMONAS TUMEFACIENS IN VITRO

This portion of the research was carried out by Dr. Annette Hervey using the method of serial dilution described in a previous publication (5). Five strains of *Ph. tumefaciens* having different cultural characteristics and different degrees of virulence were tested against seven antibacterial substances. The strains of bacteria were cultured 48 hr. in broth, diluted 1:1000, distributed in 1-ml. amounts among 10 tubes to the first of which 1 ml. of the antibiotic to be tested was added. By serial transfer of 1 ml. from tube to tube, a series of dilutions of the antibacterial substance was obtained. The tubes were incubated at 25° C. and readings were taken after 1, 2, and 3 days. The end point was taken as the last tube showing complete inhibition of bacterial growth. The concentrations of antibacterial substances which gave complete inhibition are shown in table 1.

Among some of the strains tested, big differences in sensitivity to the various antibacterial substances were observed. These differences were not correlated with the tumefacient power of the strain. The strains BP and B6, both of which were highly tumefacient, differed in their sensitivity to almost all the substances tested. Streptomycin, though active against both virulent strains, was relatively inactive against the semi-virulent strain, A6. Chloromycetin, though highly active against both the virulent strain BP and the avirulent strain SP, had little action on the other strains. Penicillin G, though it inhibited the growth of strain BP at a concentration of 4 γ per liter, was completely inactive against the other strains. The highest overall activity was shown by aureomycin. Even to this substance strain

A6 was one hundred times less sensitive than strain SP. It is not possible to determine, on the basis of present data, what factors were responsible for these great differences in strain sensitivity.

TABLE 1.—*Lowest concentrations (γ per ml.) of various antibacterial substances which inhibited growth of Phytomonas tumefaciens in broth (24-hr. readings)*

Antibacterial substance	Strain of *Phytomonas tumefaciens*				
	BP	B6	B2	A6	SP
Penicillin G	4.0	500	1000	1000	31.0
Streptomycin	1.5	4.0	0.2	50.0	12.5
Streptothrycin	0.8 unit	25 units	0.8 unit	25 units	3.2 units
Chloromycetin	0.2	31.0	31.0	2.0	0.8
Patulin	2.0	31.0	15.6	7.8	8.0
A 377 hydrochloride (aureomycin)	0.03[a]	0.06	0.025	1.0	0.01[a]
Aspergillic acid	8.0	63.0	63.0	63.0	8.0
Tolu-p-quinone	4.0	62.0	31.0	15.6	4.0

[a] (42-hr. readings)

THE GROWTH OF NORMAL AND BACTERIA-FREE TUMOR TISSUE IN THE PRESENCE OF PENICILLIN AND STREPTOMYCIN

Nutrient agar was prepared containing streptomycin, commercial penicillin, and penicillin G in concentrations of 5, 50, and 500 γ per ml. On this agar, fragments of tumor tissue of *Helianthus annuus* and *Vinca rosea* were placed. The fresh weight of each of these fragments was obtained by

TABLE 2.—*Ratio of final to initial fresh weights of normal and tumor tissue of Helianthus and Vinca rosea after 4 weeks culture in the presence of antibiotic substances (means of 20 estimates)*

Antibiotic substance	Concentration γ per ml.	Type of tissue			
		Vinca normal	Vinca tumor	Helianthus normal	Helianthus tumor
Commercial penicillin	5	5.35	4.18	17.47	6.81
	50	4.01	3.60	11.36	5.40
	500	3.00	3.71	7.82	2.85
Penicillin G	5	.....	.....	1.77	6.70
	50	.....	.....	8.50	6.00
	500	.....	.....	9.21	3.21
Streptomycin	5	3.11	4.24	2.52	7.03
	50	2.50	3.27	1.71	4.29
	500	2.41	1.73	1.78	2.12
No antibiotic	.....	3.41	5.20	1.87	6.80

weighing the culture tube before and after addition of the tissue fragment. Initial dry weights were estimated from separate samples. Tissue from the stems of normal plants was obtained as previously described. After 4

weeks at 25° C. in continuous light, the tissues were removed from the culture vessels and their individual fresh weights and pooled dry weights were determined. Twenty replicates of every treatment were used in this experiment, and the ratio of final to initial fresh weights is shown in table 2. Dry weight increases were found to be proportional to fresh weight increases and have therefore not been included.

Evidently the growth of tumor tissue of both kinds was not significantly influenced by the presence of commercial penicillin in the medium unless the concentration of this substance was of the order of 500 γ per liter. This sample of penicillin contained an amount of indoleacetic acid sufficient to cause root production in normal tissue both of sunflower and, to a lesser extent, of Vinca. The very great weight increase of normal sunflower tissue in the presence of this substance was due to this production of roots. Penicillin G had some inhibiting action on sunflower tumor tissue at a concentration of 500 γ per ml. It induced root formation at a concentration of 50 and 500 γ per ml. in normal sunflower tissue. Possibly this effect was also due to traces of indoleacetic acid. Streptomycin exerted a more powerful inhibiting action on the growth of both types of tumor tissue, being active in a concentration of 50 γ per ml. and still further reducing growth at 500 γ per ml. Even at this concentration, however, it did not inhibit the growth of the tumor tissue completely. Streptomycin reduced the growth of normal tissue to a much smaller extent than it did that of tumor tissue.

THE ACTION OF STREPTOMYCIN AND PENICILLIN ON TUMORS ON ISOLATED FRAGMENTS OF CARROT TISSUE

Cylinders of sterile carrot tissue 10 mm. diam. and 6 mm. high were placed on slopes of nutrient agar and inoculated on their upper surfaces with a suspension of *Ph. tumefaciens*, strain BP. The suspension was prepared to have a standard opacity and diluted to give an approximately known number of organisms. Preliminary experiments showed that as few as ten organisms applied to the freshly cut surface of carrot tissue would suffice to give rise to tumors which appeared as slight green protuberances about 6 days after the inoculation of the fragment. A detailed description of the structure and mode of origin of these tumors will be published elsewhere. In this series of experiments attention was focused on the effect of applications of antibiotic substances on the development and subsequent growth of these tumors.

In the first experiments of this series both the concentrations of antibiotic substances and the number of organisms in the inoculum were varied. The disks of carrot tissue were inoculated as soon as they had been placed on nutrient agar, the inoculum being evenly spread over the upper surface of the stem fragment. One day after its inoculation each fragment was treated with 0.05 ml. of a solution of antibiotic substance which was also

applied to the upper surface of the fragment. After incubation for 2 weeks in continuous light at 25° C., the number of fragments bearing tumors on their upper surfaces were counted. The results of this experiment are shown in table 3.

Both streptomycin and penicillin G were capable of reducing tumor formation in carrot fragments inoculated with *Ph. tumefaciens*, but streptomycin was far more potent in this respect than was penicillin. Thus, when the inoculum contained 50 million organisms, penicillin had no effect on tumor formation even in a concentration of 5000 γ per ml. Streptomycin at this concentration completely inhibited tumor formation, and at

TABLE 3.—*Effect of antibiotic substances on tumor formation on carrot fragments inoculated with suspensions of Phytomonas tumefaciens containing different numbers of organisms. Figures show number of fragments out of ten on which tumors were apparent at the end of 2 weeks*

No. of organisms in inoculum	Concentration of antibiotic substance (γ per ml.)				
	5000 γ	500 γ	50 γ	5 γ	0 γ
			Streptomycin		
50×10^6	0	3	10	10	10
50×10^4	1	4	10	10	10
50×10^2	2	5	10	10	10
50	0	3	6	10	10
0	0	0	0	0	0
			Penicillin G		
50×10^6	10	10	10	10	10
50×10^4	9	10	10	10	10
50×10^2	4	10	10	10	10
50	2	6	10	10	10
0	0	0	0	0	0

a concentration of 500 γ per ml. reduced the incidence of tumors from 10 out of 10 to 3 out of 10. This greater efficiency of streptomycin was apparent at all levels of the inoculum; in fact, it appears that the tumor-inhibiting action of streptomycin at the higher concentrations was about ten times more powerful than that of penicillin G.

It was observed that the tumor-inhibiting action of these antibiotic substances was limited to the upper surface of the disk to which they were applied. Even on disks treated with high concentrations of streptomycin, tumors occasionally appeared on the sides of the fragments to which the bacteria had gained access. At concentrations of 500 γ and over, streptomycin appeared to be toxic to the carrot tissue and inhibited cell proliferation both on inoculated and on noninoculated tissue.

To determine whether streptomycin was capable of causing the regression of tumors once they had formed, a series of fragments of carrot tissue was prepared, inoculated with a suspension of *Ph. tumefaciens* containing

about 5000 organisms per ml. and treated with a solution of 500 γ per ml. streptomycin 1, 2, 6, and 8 days after inoculation. The disks were incubated for 4 weeks, at the end of which time the number of fragments with tumors in each group of ten tissue fragments was recorded (Table 4).

Evidently streptomycin was capable of inhibiting tumor formation only when it was applied during the first few days after the tissue had been inoculated. When the tumors were already formed, application of streptomycin brought about a reduction in their rate of growth but did not cause their regression. This finding suggests that the action of streptomycin may be on the bacteria rather than on the tumor tissue itself, though further work is needed to make clear the manner in which this antibiotic substance exerts its effect.

TABLE 4.—*Frequency of tumor formation on carrot fragments treated with 500 γ per ml. streptomycin at different intervals after inoculation with Phytomonas tumefaciens. (Figures show number of fragments out of 10 with visible tumors)*

Treatment	Number of fragments with tumors
Untreated	10
Streptomycin applied after 1 day	1
Do 2 days	0
Do 6 days	10
Do 8 days	10

SUMMARY

Six strains of *Phytomonas tumefaciens* were grown in broth culture in the presence of the following antibacterial substances: penicillin G, streptomycin, streptothrycin, chloromycetin, patulin, aureomycin, aspergillic acid, and tolu-p-quinone. The concentrations of these substances needed to inhibit growth of *Ph. tumefaciens* varied greatly among the different strains. Aureomycin, chloromycetin, streptomycin, and streptothrycin showed the highest degree of activity.

Normal and bacteria-free tumor tissue of *Helianthus annuus* and *Vinca rosea* were cultured *in vitro* on agar containing different concentrations of commercial penicillin, penicillin G, and streptomycin. At a concentration of 500 γ per ml. both penicillin preparations had an inhibiting action on the growth of tumor tissue. The growth of normal tissue was stimulated by these preparations chiefly owing to the fact that they both induced root production, an effect probably due to traces of indoleacetic acid. Streptomycin inhibited the growth of tumor tissue more actively than did penicillin, being effective in a concentration of 50 γ per ml.

Sterile slices of carrot tissue were inoculated with *Ph. tumefaciens* and treated one day later with penicillin G or streptomycin. Tumor formation was inhibited to some extent by both these substances, streptomycin

being more powerful in this respect than was penicillin. Once the tumors had been formed they were not caused to regress by streptomycin though their rate of growth was slowed down. It appeared that the effect of the streptomycin resulted from its action on the bacteria rather than from action on the tumor cells themselves.

NEW YORK BOTANICAL GARDENS
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RESISTANCE TO EARLY BLIGHT AND SEPTORIA LEAF SPOT IN THE GENUS LYCOPERSICON[1]

SETH BARTON LOCKE[2]

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INTRODUCTION

Differences in amount of defoliation frequently have been observed among commercial tomato varieties exposed under field conditions to infection by various leaf-spotting fungi. Yet a number of investigators (1, 3, 4) have failed to discover any appreciable differences in resistance to infection by certain of these pathogens when tomato varieties were artificially inoculated under conditions which were highly favorable to infection. The author's experience in this connection has been the same. Using a laboratory technique described in detail elsewhere (10), small but statistically significant differences in susceptibility to early blight (*Alternaria solani* (E. and M.) Jones and Grout) and Septoria leaf spot (*Septoria lycopersici* Speg.) were indicated among 15 common tomato varieties. However, strains of the same variety from different sources and even individual plants from the same seed lot sometimes reacted differently. In no case was a degree of resistance indicated which was sufficiently high to affect appreciably the amount of defoliation in the field. It is thus apparent that the observed differences in amount of defoliation of tomatoes in the field are not exclusively due to differences in resistance to infection by the common leaf-spotting fungi. Such factors as growth habit, bearing on disease escape, and fruit load, affecting predisposition of the suscept to infection, enter into the picture (5, 7).

In the absence, among improved tomato types, of a source of practical resistance to early blight and Septoria leaf spot, it seemed desirable to extend the search for such resistance to include the so-called "wild" forms of *Lycopersicon esculentum* Mill. and to other *Lycopersicon* species. In 1939 a lot of seed was obtained from the Division of Plant Exploration and Introduction, Bureau of Plant Industry, which comprised the remnants of a collection made by Dr. H. L. Blood in South America in 1938. The names employed here are those published by Muller (11). This collection included

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[2] Formerly Assistant Plant Pathologist, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas; now Associate Plant Pathologist, Washington State College, Pullman, Washington.

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69 numbers embracing five *Lycopersicon* species and several forms and varieties. For the most part, only 10 or 12 seeds of each number were supplied, and in some cases only one or two plants were obtained from these. This material was subjected to laboratory tests for reaction to early blight and Septoria leaf spot early in 1940 and again early in 1941 and to a field test during the summer of 1940. A preliminary report of the results of these tests was made in 1941 (9).

REACTION TO INOCULATION WITH ALTERNARIA SOLANI

As shown in table 1, significant differences in lesion diameters resulting from inoculation with *Alternaria solani* occurred among the accessions representing the same species in all instances. However, only two accessions were significantly more resistant than other strains in their respective species groups in both the 1940 and the 1941 tests. One was a strain of *Lycopersicon hirsutum* Humb. and Bonpl., P. I. No. 127827[3,4], which was significantly more resistant than five out of six and four out of seven other strains of the same species in the 1940 and 1941 tests respectively. This strain had the smallest mean lesion diameter of any of the strains tested. The other strain mentioned, P.I. No. 126953 of *L. pimpinellifolium* (Jusl.) Mill., was significantly more resistant than 6 out of 20 and one out of 20 other strains of the same species in the 1940 and 1941 tests respectively. Its mean lesion diameter for the two tests was approximately twice that obtained with the *L. hirsutum* strain, P.I. No. 127827.

On the basis of the combined data of the two tests, the order of increasing mean lesion diameters for species was *Lycopersicon hirsutum*, *L. glandulosum* C. H. Mul., *L. esculentum*, *L. pimpinellifolium*, and *L. peruvianum* (L.) Mill.

REACTION TO INOCULATION WITH SEPTORIA LYCOPERSICI

Statistically significant differences in mean lesion diameters resulting from inoculation with *Septoria lycopersici* were obtained in all of the species groups excepting that of *Lycopersicon peruvianum* in the 1940 test, and only in *L. pimpinellifolium* in the 1941 test. The strains of the last-named species which showed significantly smaller lesions were not the same in the two tests in any instance. In the *L. hirsutum* group the significant difference in the 1940 test occurred between one strain, P. I. No. 126444, which produced a large lesion, and the remaining six strains all of which produced small lesions and which were not significantly different from one another in this respect. The more susceptible strain was not included in the 1941 test. With the exception of P. I. No. 126444, the mean lesion diameters obtained with *L. hirsutum* strains were smaller than that obtained with any other accession tested.

[3] P.I. No. refers to the accession numbers of the Division of Plant Exploration and Introduction.

[4] P.I. No. 127827 was represented in these tests by a vegetatively propagated clone derived from a single plant. This species failed to produce seeds under the conditions which prevailed during these studies.

TABLE 1.—*Reaction of South American Lycopersicon importations to infection with Alternaria solani and Septoria lycopersici*

Species	P.I. No.[a]	Mean lesion diameter[b]				Defoliation[c]
		A. solani		*S. lycopersici*		*S. lycopersici*
		1940	1941	1940	1941	1940
		mm.	*mm.*	*mm.*	*mm.*	*Per cent*
L. hirsutum	126444	5.74	.....	1.76	.....	35.8
	126445	4.74	6.30	0.16	0.10	9.0
	126446	4.16	4.92	0.12	0.00	10.0
	126447	3.36	5.56	0.08	0.16	15.0
	126449	3.82	6.48	0.08	0.22	.....
	126936	5.32	.....	0.16	.....	30.0
	127826	.....	7.32	.....	0.40	15.0
	127827	1.40	3.72	0.04	0.00	13.0
f. *glabratum*	134417	.....	6.62	.....	.....	18.0
	134418	.....	7.30	.....	0.22	12.5
Species mean		4.08	6.03	0.34	0.16	17.6
L.S.D.[d]	(19:1)	2.39	2.64	0.36	2.92	
	(99:1)	3.45	.....	0.53	.....	
L. glandulosum	126438	3.48	.....	1.28	.....	21.0
	126439	5.44	.....	0.48	.....	21.6
	126440	5.14	.....	0.72	0.44	51.6
	126441	.....	.....	0.18	.....	.....
	126443	5.28	.....	.....	.....	37.5
	126448	3.38	.....	0.32	.....	13.0
Species mean		4.54	.....	0.60	0.44	28.9
L.S.D.	(19:1)	2.02	.....	0.74	.....	
	(99:1)	.....	.....	1.08	.....	
L. peruvianum	126926	3.56	7.20	1.18	1.28	.....
	126928	5.12	7.50	0.34	1.56	19.0
	126929	.....	7.04	.....	1.84	15.8
	126930	4.02	8.08	0.56	1.68	31.6
	126944	5.24	8.20	1.42	1.58	12.5
	126945	4.94	8.64	1.28	1.06	35.1
	126946	5.16	8.32	1.08	0.74	25.0
var. *dentatum*	126935	.....	7.96	.....	1.80	28.3
	127830	.....	.....	0.46	.....	31.0
	127831	.....	.....	0.94	.....	27.0
	127832	5.18	7.80	1.10	2.32	25.0
var. *humifusum*	127828	.....	6.48	.....	1.62	17.5
	127829	5.97	7.40	1.02	1.60	14.6
Species mean		4.90	7.67	0.94	1.55	23.5
L.S.D.	(19:1)	1.64	2.15	0.98	1.26	
	(99:1)	.....	.....	.....	.....	
L. esculentum	126451	.....	7.14	.....	1.06	33.3
	127815	4.94	7.34	0.54	1.88	21.6
	127816	5.26	6.44	0.62	.....	38.3
var. *cerasiforme*	126417	5.54	5.78	0.70	1.04	31.6
	126418	.....	5.80	.....	1.98	46.6
	126419	5.52	.....	1.40	.....	46.6
	126425	5.08	6.32	.....	0.80	21.0
	126426	.....	6.90	.....	1.32	20.0
	126942	3.68	5.78	.....	1.56	30.8

TABLE 1. (*Continued*)

Species	P.I. No.[a]	Mean lesion diameter[b] *A. solani* 1940	*A. solani* 1941	*S. lycopersici* 1940	*S. lycopersici* 1941	Defoliation[c] *S. lycopersici* 1940
		mm.	*mm.*	*mm.*	*mm.*	*Per cent*
	126950	.....	6.38	1.62	1.70	31.0
	127814	4.20	.....	0.66	.....	9.1
var. *pyriforme*	126452	4.60	.....	0.20	.....	27.5
	126955	6.42	5.46	1.12	1.58	39.1
Species mean		5.03	6.33	0.86	1.44	29.0
L.S.D.	(19:1)	1.88	1.68	0.96	1.32	
	(99:1)	2.73	.....	1.40	.....	
L. pimpinellifolium	126432	4.96	7.66	0.92	1.68	50.8
	126433	5.34	6.82	.....	1.78	32.0
	126436	6.10	6.10	.....	1.86	22.0
	126437	.....	6.58	.....	2.26	45.0
	126925	5.84	8.38	1.14	.....	15.8
	126927	.....	7.18	1.58	2.10	35.0
	126931	.....	6.94	.....	1.28	47.5
	126932	4.56	.....	0.62	.....	43.3
	126933	4.54	7.16	1.14	1.56	48.3
	126934	5.08	.....	1.28	.....	39.1
	126937	4.47	.....	1.80	.....	36.2
	126938	.....	6.70	.....	2.64	40.0
	126939	4.96	.....	0.74	.....	26.0
	126940	5.48	7.80	0.78	1.52	45.0
	126941	4.82	.....	0.68	.....	16.0
	126943	4.82	7.42	1.52	1.50	18.7
	126947	5.76	7.54	2.54	.....	45.8
	126948	3.76	7.56	.....	2.80	43.3
	126950	4.52	.....	.....	.....	.....
	126951	4.18	7.58	1.46	0.74	57.6
	126952	5.18	8.32	.....	0.62	16.2
	126953	3.76	6.52	1.50	0.72	50.8
	126954	.....	7.60	.....	1.84	40.0
	127805	3.92	6.68	1.56	2.38	21.6
	127806	4.72	.....	2.08	.....	21.6
	127807	6.02	7.28	1.78	2.24	
	127833	5.52	6.70	.....	2.38	25.0
Species mean		4.99	7.33	1.36	1.77	34.9
L.S.D.	(19:1)	1.67	1.81	1.20	1.29	
	(99:1)	2.40	.....	1.74	1.88	

[a] Accession numbers of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. D. A.
[b] Laboratory tests.
[c] Field test.
[d] Least difference required for significance.

In both tests the order of increasing mean lesion diameter for species was *Lycopersicon hirsutum*, *L. glandulosum*, *L. esculentum*, *L. peruvianum*, and *L. pimpinellifolium*. In both tests *L. hirsutum* was significantly more resistant than all of the other species excepting *L. glandulosum*. Significant differences occurred among the remaining species groups in both tests, but they were not consistent in the two tests.

DEFOLIATION IN THE FIELD

Field plantings of most of the numbers included in the laboratory tests, made in 1940 at the main station farm near Fayetteville, Arkansas, afforded an opportunity for comparing the results of artificial inoculation with the amount of defoliation occurring under natural conditions. Single plots of six plants each were arranged at random in the planting. Where fewer than six seedlings of a strain were available, the strain was represented by a clone of vegetatively propagated individuals. This was the case with the *Lycopersicon hirsutum* strains excepting two belonging to the form *glabratum* C. H. Mul., most of the *L. glandulosum* strains, and several *L. peruvianum* strains.

Early blight appeared in planting early in the season, but failed to develop sufficiently to account for appreciable defoliation. Septoria leaf spot, however, became severe, and toward the end of the season all susceptible strains were badly defoliated. On September 10, the percentage of defoliation was estimated for each plant, working across the plots in order to minimize the personal factor. An average percentage of defoliation for each accession, based upon six independent observations, appears in the right-hand column of table 1.

No true replications of strains were provided in the field test, since the six plants of each strain were placed together in a single plot. Consequently, differences between individual strains are apt to be overshadowed by effects associated with location in the planting. However, legitimate comparisons between species can be made because the individual strains comprising them were scattered at random over the planting.

With the exception of *Lycopersicon glandulosum*, the field defoliation data parallel the laboratory data for resistance to Septoria leaf spot. *L. hirsutum* showed the least defoliation and *L. pimpinellifolium* the most. The three remaining species, *L. glandulosum*, *L. esculentum*, and *L. peruvianum*, showed only slightly less defoliation than *L. pimpinellifolium*. The apparent susceptibility of *L. glandulosum* in the field test, in contrast to its apparent resistance in the laboratory test, may be associated with its lack of vigor, which may have been a contributing factor to its defoliation in the field.

DISCUSSION

Before drawing conclusions from the data presented here, it seems appropriate to compare them with those obtained by other investigators working with the same or similar material. In 1938, Butler (6) reported briefly on the amount of defoliation occurring in 265 *Lycopersicon* importations under field conditions in Tennessee. *Septoria*, *Alternaria*, and *Cladosporium* were mentioned as contributing to defoliation, but the relative importance of each in this respect was not stated. In 1942, Alexander, Lincoln, and Wright (2) reported on field tests in Ohio and Indiana for resistance to early blight and Septoria leaf spot in 488 *Lycopersicon* importations.

Andrus and Reynard reported in 1942 on laboratory and field tests for resistance to early blight (3) and in 1945 on laboratory tests for resistance to Septoria leaf spot (4).

Butler found that four strains of *Lycopersicon hirsutum* were almost free from leaf spots in his test (6). The Ohio and Indiana field tests indicated a high degree of resistance in ten lines of *L. hirsutum* toward both Septoria leaf spot and early blight (2). Andrus and Reynard reported a high degree of resistance to Septoria leaf spot in several *L. esculentum* × *L. hirsutum* hybrids (4). On the basis of field observations they report that *L. hirsutum* is resistant to early blight (3). The results of the laboratory and field tests reported here are in agreement with those of other workers (2, 3, 6) insofar as reaction to Septoria leaf spot is concerned. No field test for reaction to early blight was obtained in Arkansas, but the author's laboratory tests indicated that only one accession of *L. hirsutum*, P. I. No. 127827, showed marked resistance to early blight. Considerable variation exists among individual plants of a strain represented by a single P. I. number. Consequently, the material tested by different investigators probably was not genetically identical. With the exception of P. I. No. 127827, it also is highly probable that resistance toward early blight observed under field conditions in strains of *L. hirsutum* is associated with the high degree of sterility shown by this species in North America. Extent of fruit load has been shown to affect susceptibility to defoliation diseases in tomatoes to a marked degree (5, 7). In the writer's laboratory tests where fruit load was not a factor, P. I. No. 127827 was resistant to early blight.

Alexander *et al.* (2) state that, from limited field observations, *Lycopersicon glandulosum* did not appear to possess any resistance to the diseases studied. However, they rate (in their table 3) one strain, P. I. No. 126448, of this species as resistant to Septoria leaf spot and early blight in their field tests. This particular accession showed some resistance to Septoria leaf spot and early blight in the writer's laboratory and field tests (Table 1). As pointed out earlier, *L. glandulosum* is not so vigorous as the other species. Consequently it may appear to be susceptible in field defoliation tests.

Strains of *Lycopersicon peruvianum* have been judged resistant to one or the other of the diseases in question by each of the workers testing them. However, there appears to be no agreement with regard to the particular accessions which are resistant. In the Indiana report, many lines are listed as heterozygous with respect to their reaction to the two diseases. This may be the explanation for a great deal of the confusion surrounding the reactions of this group.

In the case of *Lycopersicon peruvianum* var. *humifusum*, a different explanation is possible. Although the field tests in Arkansas, Ohio, and Indiana indicate resistance in strains of this variety, the writer's laboratory tests indicate that they are susceptible to both diseases. As observed in Arkansas, plants of this variety are extremely open in growth habit, pro-

ducing long, trailing vines with widely spaced leaves. This growth habit obviously is less favorable for infection than the dense type because it permits rapid drying of the foliage after rain- or dewfall. The writer is inclined to look upon the lack of defoliation of this variety in the field as disease escape rather than disease resistance. This view is supported by results obtained by Wright and Lincoln (12) who crossed a strain of this variety, P. I. No. 127829, with the tomato variety, Indiana Baltimore. They reported in 1940 that resistance was recessive in the F_1 generation. In 1941, however, Lincoln (8) reported that all of approximately 2,200 F_2 plants from this cross were susceptible to early blight and Septoria leaf spot. It thus appears that, as soon as the open habit of *L peruvianum* var. *humifusum* was lost, the apparent field resistance to defoliation diseases was lost also.

Butler (6) found one large-fruited strain of *Lycopersicon esculentum* (P. I. No. 128285) from Argentina, which showed only a small percentage of defoliation in the field. Alexander *et al.* (2) rated this accession as susceptible to Septoria leaf spot in their field tests. Andrus and Reynard (4) tested several other lines showing resistance to Septoria leaf spot which, they considered, were of hybrid origin, that is, outcrosses of *L. esculentum* with either the currant or cherry tomato types, and with *L. hirsutum*. One strain of *L. esculentum* var. *cerasiforme* (P. I. No. 127814) showed some resistance to defoliation in the field in Arkansas but rated susceptible in the writer's laboratory tests and as moderately resistant in the Ohio field test (2).

Alexander *et al.* (2) list 14 accessions of *Lycopersicon pimpinellifolium* as moderately resistant to Septoria leaf spot, as indicated by small lesion size. Andrus and Reynard (3) place 13 out of 14 accessions of this species in their more resistant classes with respect to early blight. They consider these as significantly tolerant to this disease. They noted outstanding resistance to defoliation in the field in progenies of accession P. I. No. 79532. This accession was not tested by the writer, but it is listed as susceptible to both early blight and Septoria leaf spot in the Indiana report (2). Of the 27 *L. pimpinellifolium* accessions tested by the writer only two showed resistance to Septoria leaf spot in both laboratory and field tests. One of these, P. I. No. 126925, was rated in the Indiana report (2) as moderately resistant. The other, P. I. No. 126952, was listed in the same report as susceptible to this disease.

It thus appears that considerable variability exists in the material tested under the same accession numbers by different investigators. However, there is general agreement with respect to the resistance of *Lycopersicon hirsutum* accessions to Septoria leaf spot. The writer believes that his tests indicate accession P. I. No. 127827 of this species as possessing the highest degree of resistance to both early blight and Septoria leaf spot found in any of the material tested. The probable value of this strain of *L. hirsutum* for breeding disease-resistant tomato varieties will be discussed in a subsequent publication.

SUMMARY

Small differences in susceptibility to early blight and Septoria leaf spot were found among 15 common tomato varieties by means of artificial inoculation. Sixty-nine *Lycopersicon* importations from South America were tested in the laboratory and field in search of a source of resistance for breeding purposes. Some degree of resistance was found in accessions belonging to each of the five species represented. The highest degree of resistance to either disease was found in strains of *L. hirsutum*, and one accession, P. I. No. 127827, was found to possess a high degree of resistance to both diseases.

UNIVERSITY OF ARKANSAS
AGRICULTURAL EXPERIMENT STATION
FAYETTEVILLE, ARKANSAS

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WISCONSIN PEA STREAK[1]

D. J. Hagedorn and J. C. Walker

(Accepted for publication July 6, 1949)

Linford (6) in 1929 reported that the streak disease of pea (*Pisum sativum* L.) was widespread in the United States, but no description of a causal agent was given until 1938 when Zaumeyer (13) discussed the relationship of pea streak to several strains of the alfalfa mosaic virus. He named the virus inciting this disease pea streak virus 1. The following year another pea streak virus was described by Chamberlain (1) in New Zealand.

In Wisconsin, pea streak was evidently not noticeable in 1924 because Jones and Linford (5) made no mention of it when they reported on the pea disease survey made that year. However, in the same State in 1942 Walker and Hare (10) found pea streak in about 60 per cent of 654 pea fields surveyed and in an occasional field the damage was severe. In a study of pea virus incidence in Wisconsin in 1948, pea streak was found to be widespread in all of the 12 important pea-growing counties surveyed.

A study of the nature of the pea streak disease, typical of that found in Wisconsin pea fields, was begun in 1946.

METHODS AND MATERIALS

For greenhouse studies plants were grown in compost soil in 6-in. earthenware pots at temperatures from 20° to 24° C. Plants of Perfected Wales, a very susceptible variety, were used for maintenance of stock cultures and most experiments. The virus was transmitted readily by rubbing young but fully expanded leaves, which had been sprinkled lightly with carborundum powder, with a pad made by folding cheesecloth around macerated diseased tissue. Determinations of physical properties were made in a manner similar to that described by Johnson and Grant (4), the exception being that aging tests were conducted at a temperature of 22° C. (72° F.) instead of at 80° – 90° F.

Virus-free pea aphids (*Illinoia pisi* (Kalt.)) were maintained on healthy broad beans (*Vicia faba* L.) or on peas in an aphid-tight wire mesh cage. Similar cages were used for insect detention during tests.

Six to 14 aphids were placed on each plant, and were confined to the plants by glass lamp chimneys. In most cases aphids were transferred by removal of the leaf on which they were feeding to the desired location.

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When close study was to be made of feeding periods, aphids were moved by use of a camel's-hair brush.

EXPERIMENTAL RESULTS

Symptoms on Pea

Pea streak is a very conspicuous disease in the field. The most obvious

FIG. 1. Pea plant showing symptoms of Wisconsin pea streak as seen in the field. Note stem and petiole streaking, failure of pods to fill, dorsal suture discoloration, chlorosis and wrinkling of apical foliage.

symptom is necrosis of the stem and petioles (Fig. 1). Light brown to purple, oblong, necrotic lesions of various sizes are scattered along the surface of these plant parts. A single large lesion, formed by the coalescence

of several small ones, sometimes extends the length of several internodes. Many times the stem is girdled by these necrotic areas. Streaking is often present on the peduncle, and the dorsal suture of the pod sometimes shows a brownish discoloration. The nodes are also discolored. Irregularly

FIG. 2. Symptoms of Wisconsin pea streak in the greenhouse. Plant on left healthy; others diseased. Note progressive wilting and slight reduction in size of diseased plants.

shaped, light brown, slightly sunken, necrotic areas occur on both leaves and pods giving them a roughened appearance. If plants are attacked when young, the pods which do develop fail to fill normally. Terminal

plant parts are slightly malformed as a result of internode shortening and apical leaf wrinkling and curling. A mild general chlorosis of the leaves on the upper half of the plant is often observed but neither vein clearing nor mottle is characteristic. Moderate axillary bud growth is common. Some plants die prematurely.

Symptoms of pea streak were much less severe in the greenhouse than in the field. A progressive wilting of the plant (Fig. 2) was more characteristic in the greenhouse. The first noticeable symptom was a steel-gray discoloration of the stem. At this time the nodes above the inoculated leaves showed a slight browning. A few days later the inoculated leaves were dried but remained attached and their stipules were wilting. The first set of leaves and stipules above those inoculated was chlorotic and beginning to wilt. As wilting of the foliage progressed upward, the stipule became chlorotic and wilted before the leaf at the same node. In some cases, the uppermost leaves and stipules had become chlorotic 11 to 14 days after inoculation. At this time small, brown, necrotic streaks could sometimes be seen in the veins of leaves and stipules and on the stems. The browning at the nodes was conspicuous and the entire stem was grayish-tan. After 14 or 15 days, many plants had wilted completely and were dead. On others the stem streaks were from a few millimeters to several centimeters in length, even extending from one internode into another. The upper two sets of leaves and stipules were still turgid, but all other foliage had wilted. Stunting of infected plants was apparent occasionally. This symptom was caused, in part, by a slight reduction of internode length, but mainly by failure of the plant to continue its growth normally. A curling over of the apical growth sometimes occurred a few days before complete wilting and death. No vein clearing or mottle was observed.

Symptoms on Other Legumes

The Wisconsin pea streak virus incited vein clearing, and later mosaic symptoms on red clover (*Trifolium pratense* L.). Chlorosis along the main vein was conspicuous and small splotches of dark green tissue were dispersed irregularly over the mottled leaflets (Fig. 3, A). On alsike clover (*Trifolium hybridum* L.) vein clearing was the first symptom and later faint, small, oblong, chlorotic areas developed along the veins and between them, giving a somewhat mottled effect (Fig. 3, B). Infected leaves of this host were slightly wrinkled. Infected white sweet clover (*Melilotus officinalis* (L.) Lam.) displayed symptoms of mosaic—a few dark green splotches and chlorotic areas with definite margins scattered over the leaflet (Fig. 3, E). Symptoms on yellow sweet clover (*M. alba* Desr.) were vein clearing, mottle caused by interveinal chlorotic spots of various sizes and shapes, and a general loss of green color throughout the entire leaf (Fig. 3, D). On Ladino white clover (*Trifolium repens* L.) the only

symptom was a very slight leaf crinkle. Infected soybeans (*Soja max* (L.) Piper) showed very faint general chlorosis.

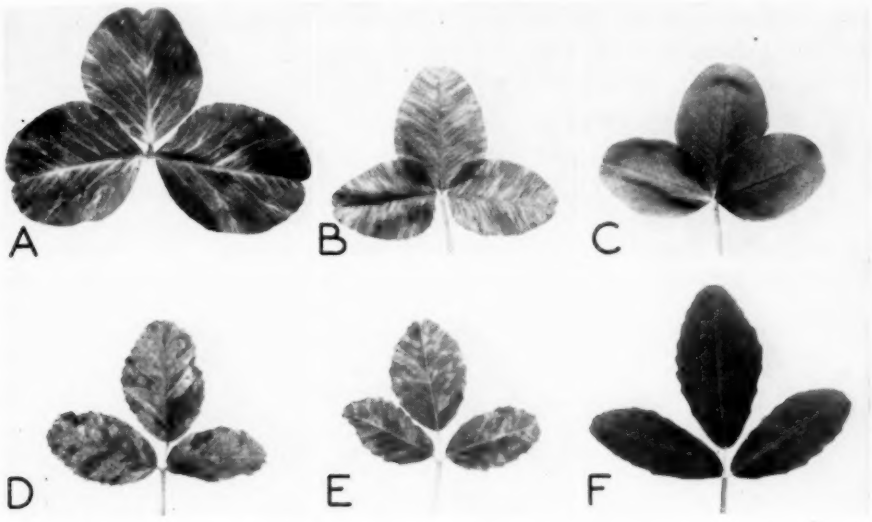

FIG. 3. Symptoms of the Wisconsin pea streak virus on clover leaves. A, diseased red clover; B, diseased alsike clover; C, healthy alsike clover; D, diseased white sweet clover; E, diseased yellow sweet clover; F, healthy white sweet clover.

Effect of Temperature upon Disease Development

The effect of air temperature upon disease development on pea was studied at 16°, 20°, 24°, and 28° C. At 16° C. symptoms did not appear until 25 days after inoculation. Brown streaks on the stem were much more severe at this temperature than at higher temperatures and death from progressive wilting was delayed as long as 45 days after inoculation. When inoculated plants were grown at 20° C., initial disease symptoms could be seen as early as the seventh day and plants often were dead 10 days later. Symptoms at this temperature were similar to those previously described. Inoculated plants growing at 24° C. and 28° C. developed typical symptoms in seven days. Death occurred 15 days after inoculation at the former temperature and in 12 days at the latter. At the two highest temperatures progressive wilting of the diseased plants was the most conspicuous symptom, while stem streaking and node browning were very slight or absent.

Host Range

Twenty-four species of the Leguminosae and six from other families were tested for susceptibility to the Wisconsin pea streak virus. Plants studied and results obtained are given in table 1. Recoverability of the virus from host plants was tested by inoculation back to healthy Perfected Wales peas. Although no symptoms were observed when the virus was

recovered, the degree of susceptibility was designated as "latent." If symptoms were absent or questionable and the virus was not recovered, the host was considered nonsusceptible. The virus was found to be limited in host range to the legumes, 17 species of which were infected. It was not infectious to cucumber (*Cucumis sativus* L.), jimsonweed (*Datura stramonium* L.), tomato (*Lycopersicon esculentum* Mill.), or any of the three species of *Nicotiana* tested.

TABLE 1.—*Host range and symptoms of the Wisconsin pea streak virus*

Host	Plants inoculated	Plants diseased	Outstanding symptom
	Number	*Number*	
Lathyrus hirsutus L.	18	Latent[a]	
L. odoratus L.	12	Latent	
Lupinus angustifolius L.	7	0[a]	
Medicago orbicularis All.	5	Latent	
M. sativa L.	17	0	
Melilotus alba Desr.	22	6	mottle
M. officinalis (L.) Lam.	22	9	mottle
Phaseolus vulgaris L.	32	0	
Pisum sativum L.	40	32	stem streak, plant wilt
P. sativum var. *arvense* Poir.	9	4	Do
Soja max (L.) Piper	31	12	very slight general chlorosis
Trifolium hybridum L.	24	13	faint mosaic
T. incarnatum L.	17	Latent	
T. pratense L.	40	32	mottle
T. repens L. (Ladino white clover)	22	2	very slight leaf wrinkle
T. repens L. (white clover)	18	0	
Trigonella foenum graecum L.	11	Latent	
Vicia atropurpurea Desf.	16	Latent	
V. faba L. var. *major*	9	0	
V. faba L. var. *minor*	14	0	
V. grandiflora Scop.	9	2	stem streak
V. monantha (L.) Desf.	10	6	Do
V. sativa L.	11	0	
V. villosa Roth.	9	Latent	
Vigna sinensis Endl.	18	0	
Cucumis sativus L.	37	0	
Datura stramonium L.	25	0	
Lycopersicon esculentum Mill.	7	0	
Nicotiana glutinosa L.	18	0	
N. rustica L.	4	0	
N. tabacum L.	14	0	

[a] Latent = no symptoms, but virus recovered; 0 = no symptoms and no recovery.

Reaction of Varieties of Pea and Bean

Eighteen pea varieties and twelve bean varieties were tested for their reaction to the Wisconsin pea streak virus. The varieties studied and the degree of their resistance or susceptibility are presented in table 2. In several tests with Wisconsin Perfection, it was found that no infection was obtained unless inoculation was made when the plants were very young—before the second set of leaves unfolded. All of the pea varieties tested were susceptible, and the virus was easily recovered from each of them. Diseased plants of Delwiche Commando, Horal, Loyalty, Merit, Pride,

Profusion, Wasatch, and Wisconsin Perfection showed more stem streaking and less early and permanent wilting than the other varieties tested. Symptoms were not induced on any of the bean varieties tested and in no case was the virus recovered.

Properties of the Virus

A series of experiments was conducted to determine the following physical properties of the virus: tolerance to dilution, resistance to aging *in vitro*, thermal inactivation, and longevity in frozen plant tissue. Table 3 shows the results obtained. This virus displayed a rather marked tolerance to dilution. In one trial it was found still infective after a dilution of 1:100,000; two subsequent trials showed that it could be diluted to

TABLE 2.—*Varietal reaction of pea and bean to the Wisconsin pea streak virus*

Pea variety	Plants inoculated	Plants diseased	Bean variety	Plants inoculated	Plants diseased
	Number	*Number*		*Number*	*Number*
Alaska	35	27	Bountiful	7	0
Alderman	28	7	Dwarf Horticultural	11	0
Bonneville	20	10	Great Northern U. of I. No. 1	8	0
Canner King	20	14			
Delwiche Commando	16	7	Great Northern U. of I. No. 59	5	0
Glacier	23	13			
Horal	35	15	Idaho Refugee	4	0
Improved Penin	13	6	Michelite	8	0
Loyalty	17	5	Pinto	11	0
Merit	19	17	Red Kidney	32	0
Perfected Wales	39	28	Sensation Refugee 1066	9	0
Pride	16	5	Stringless Blue Lake (Black)	4	0
Profusion	22	10			
Resistant Famous	31	22	Stringless Blue Lake (White)	4	0
Thomas Laxton	28	12			
Wasatch	15	3	Stringless Green Refugee	7	0
Wisconsin Early Sweet	28	21			
Wisconsin Perfection	19	2			

1:1,000,000 and still cause infection. One experiment indicated that the virus was still infectious after aging for eight days. In the other trials, when aging studies were lengthened to include 64 days, it was found that this virus withstood aging between 16 and 32 days. All three thermal inactivation trials showed that the virus was inactivated after a 10-min. exposure at 60° C. The thermal inactivation point was somewhere between 58° C. and 60° C. When diseased plants were frozen for periods of up to 160 days, one trial showed that this virus could withstand 40 days of freezing, while the two other trials indicated survival up to only 10 and 20 days, respectively.

Insect Transmission

The possibility of the ability of the pea aphid (*Illinoia pisi* (Kalt.)) to transmit the virus was studied in the greenhouse. In all these studies the

aphids, after feeding on diseased plants, were allowed a 2-day feeding period on healthy plants. No infection was obtained with the pea streak virus in any of 5 trials, but tests made simultaneously with pea mosaic and pea stunt viruses yielded successful transmission. No transmission was secured in further tests in which aphids were allowed to feed on diseased plants for periods ranging from 15 min. to 21 days. Starving aphids

TABLE 3.—*Physical properties of the Wisconsin pea streak virus*

Property test	*Trial 1*		*Trial 2*		*Trial 3*	
	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased
Dilution:						
0	12	5	22	21	10	8
1: 10	22	15	22	21	26	22
1: 100	26	17	20	17	25	4
1: 1,000	20	6	23	15	19	10
1: 10,000	23	0	24	3	28	2
1: 100,000	25	1	18	0	25	1
1: 1,000,000	—	—	22	2	20	5
Aging *in vitro:* (days)						
0	10	2	11	5	11	8
1	11	8	—	—	—	—
2	12	5	9	4	10	4
4	11	4	10	5	9	4
8	12	1	9	3	11	9
16	—	—	10	2	9	7
32	—	—	—	—	10	0
64	—	—	—	—	11	0
Heat: (° C.) (10 min.)						
0	24	17	9	6	9	4
54	—	—	—	—	10	4
56	23	5	11	1	9	3
58	24	1	11	2	9	3
60	26	0	11	0	10	0
62	22	0	12	0	9	0
64	25	0	10	0	11	0
Freezing: (days)						
0	10	10	10	10	10	7
5	7	6	10	3	10	7
10	10	0	10	3	9	5
20	10	4	—	—	6	2
40	10	1	—	—	11	0
80	13	0	—	—	10	0
160	11	0	9	0	—	—

for 4 and 8 hr. before feeding on diseased plants likewise had no effect. In one such test the insects were starved for 4 hr. and allowed a 2-day feeding period before transfer to healthy plants. After the 8-hr. starving period, aphids were allowed to feed for 15 min., 45 min., 1½ hr., and 12 hr. before removal to healthy plants. These manipulations also yielded negative results, as did transmission tests when winged aphids were used. Limited tests with the peach aphid (*Myzus persicae* (Sulzer)) also gave negative results.

When field transmission of the virus was to be studied, Perfected Wales peas were grown in 6-in. rows in a 12×24-ft. plot, and mechanical inoculation of one plant near the center of plot was made. Fourteen pea aphids were placed on this plant when streak symptoms first became apparent. At this time two naturally occurring pea streak infection centers were noted toward the eastern end of the plot. Each consisted of five or six diseased plants. No other virus disease symptoms could be seen, even on the plants adjacent to the one mechanically inoculated. Twenty-one days after inoculation the mechanically inoculated plant was dead. Plants within a radius of 6 ft. of this inoculum plant were closely examined, but did not show a higher percentage of infection than those in the outlying areas of the plot where random counts revealed from 8 to 13 diseased plants per 100 examined. Two exceptions were noted, *viz.*, the natural infection centers mentioned above, and an area about 4×16 ft. along the southern edge of the plot where only one out of 100 plants was diseased. Twelve days later random counts were made at each end of the plot. At this time 21 plants per 100 were diseased at the western end, and 29 plants per 100 were infected at the opposite end. Pea aphids were abundant throughout the plot at all times. The distribution of the diseased plants in the plot did not indicate that the virus was spread by the pea aphid, but it did indicate clearly that probably some insect was responsible for its distribution.

Seed Transmission

Only limited seed transmission trials were possible because most pea plants infected with the virus developed only distorted pods containing very few viable seeds or were killed before pods matured. Plants were grown from 211 seeds collected from diseased plants. Under optimum greenhouse conditions for disease development, no symptoms developed on any of these plants.

DISCUSSION

To support the conclusion that the Wisconsin pea streak virus is different from previously reported pea streak viruses, a comparison will be made of the experimental evidence just presented with former descriptions of other viruses inciting pea streak.

The American pea streak virus reported by Zaumeyer (13) in 1938 differs considerably from the virus just described. Zaumeyer's virus, pea streak virus 1, showed less resistance to aging *in vitro* (2 days), a higher thermal inactivation point (65° C.), and lower tolerance to dilution (1:5,000); and it was infectious to white clover, alfalfa, and broad bean but not to sweet pea. The foliage distortion, apical rosette, and vein prominence described as symptoms were not observed on plants infected with the Wisconsin streak virus. Pea streak virus 1 was transmitted by the pea aphid, but no success was achieved in attempts to transmit the Wisconsin virus by that means.

Chamberlain (1) described the New Zealand pea streak virus in 1939 and called it *Pisum* virus 3. It differs from the Wisconsin pea streak virus in symptom expression (local lesions on inoculated pea leaves, apical plant wilt); in resistance to aging *in vitro* (41 days); in thermal inactivation point (80° C.); and in host range, since it was infectious to blue lupine, kidney bean, white clover, and cucumber.

The pea wilt virus described by Johnson (3) is similar to the Wisconsin pea streak virus in physical properties, but differs in symptoms on peas, in being infectious to kidney bean, white clover, cowpea, and broad bean, and in being noninfectious to soybean.

Other viruses have been found to produce necrosis and streaking of peas, but they differ from the Wisconsin pea streak virus in symptom expression and in host range. In this category the following may be noted: cucumber virus strains 14 and 17 of Whipple and Walker (12); tobacco ringspot virus (9); tomato spotted wilt virus (7, 8, 11).

One of the striking aspects of the Wisconsin pea streak virus is its wide annual prevalence in Wisconsin pea fields in spite of the fact that the pea aphid is not a vector. Since red clover, Ladino white clover, white sweet clover, and yellow sweet clover are susceptible, there is no lack of possible overwintering reservoirs. Further study is needed to determine the means of its current-season dissemination.

SUMMARY

Wisconsin pea streak and the causal virus are described. The most obvious symptoms on pea in the field are a necrotic streaking of the stems and petioles and node browning. The characteristic symptoms in the greenhouse were browning at the node and wilting of the plant. Low temperature (16° C.) delayed symptom expression, and necrotic stem streaking and node browning were more typical at that temperature, while wilting was the most striking symptom at 24° and 28°. The virus was limited to the Leguminosae. All 18 pea varieties tested were susceptible but none of the 11 bean varieties inoculated was infected. The virus was still infective at a dilution of 1:1,000,000 and resisted aging *in vitro* between 16 and 32 days. Its thermal inactivation point was between 58° and 60° C. and it could withstand freezing *in vivo* for 40 days. The pea aphid was not found to be capable of transmitting the virus, and seed transmission was not observed.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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STIMULATED PYCNIDIUM PRODUCTION AND SYMPHOGENOUS PYCNIDIA IN PHOMA LINGAM[1]

O. H. Calvert and Glenn S. Pound[2]

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During a study of induced variability in the Puget Sound strain of *Phoma lingam* (Fr.) Desm. (2), an unusual phenomenon occurred in a week-old Petri plate containing several single-spore colonies derived from non-treated spores of the original isolate described by Pound (7). Heavy lines of pycnidia developed between neighboring colonies of rather strikingly different types. One of these types, herein referred to as type A, was characterized by a comparatively slow growth rate, moderate to scant aerial mycelium, and few or no pycnidia. The other type, herein referred to as type B, was characterized by a fast growth rate, abundant aerial mycelium, and heavy production of pycnidia, usually in clumps. Later, many induced mutants of the Puget Sound strain were found to agree with type A in their reaction when paired with the original A and B isolates. The type A cultures differed considerably among themselves, whereas the type B cultures were all very similar and essentially unchanged from the original isolate of the strain. Since all B cultures were identical with the original isolate and gave no reaction when paired with it, the A cultures are all considered as mutants. No natural variability of the Puget Sound strain of *P. lingam* was observed by Pound (7), but the occurrence of the A type in a transfer of the original culture (which was derived from a single uninucleate spore) can only be explained as due to mutation. Several isolates of each type were studied in an effort to determine the cause of the heavy pycnidium production in the merger zone between colonies of type A and type B. From the data presented herein, it is thought that a symphogenous production of pycnidia (pycnidia produced from hyphal branches of several mycelial threads) occurs when A and B mycelia merge, but that meristogenous production of pycnidia (pycnidia produced from a single cell or adjacent cells of a single hypha) occurs within each mycelium.

MATERIALS AND METHODS

Of the isolates studied, eight mutant isolates (A2, A5, A6, A7, A14, A15, A18, A19) and four normal isolates (B2, B5, B14, B21) originated from single conidia taken from the untreated spore suspension of the original

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[2] Formerly Research Assistant, and Associate Professor of Plant Pathology, respectively, Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

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strain. Six other mutant isolates (A3, A4, A9, A10, A12, A22) and two normal isolates (B12, B22) originated from single conidia taken from a spore suspension treated for 2 min. with a 0.02 mM per ml. concentration of nitrogen mustard, methyl-bis (beta-chloroethyl) amine.

Isolates were grown on potato-dextrose agar, a single lot of agar being made for each experiment. Germinating single spores were picked with a needle from water-agar plates under a stereoscopic microscope. In the pairing experiments, single spores of monoconidial lines were used. In assaying the population of single pycnidia, each pycnidium was crushed in a drop of sterile water, and a loop of the suspension was transferred to 40 ml. of water which was then poured over the surface of agar plates. When the spores were germinating they were lifted, individually, and transferred to test-tube slants. After a few days they were separated into A and B types, according to their cultural type. As a confirmation of the type diagnosis, representatives were plated out in pairs to observe the reaction between them. In addition, some plates seeded with the spore suspension from single pycnidia were allowed to incubate so that the reaction between neighboring colonies could be observed. Confirmation tests showed that, in all cases, the separation of the spores into the two groups was accurate.

RESULTS OF PAIRING A- AND B-TYPE ISOLATES

The 20 cultures of the A and B types were paired in all possible combinations. Eight transfers, in regular patterns, were made to Petri dishes so that each isolate was grown beside each of the other isolates. These data show that A-type isolates, although they varied considerably among themselves (Fig. 1) in regard to growth rates, sporulation, pigmentation, *etc.*, were constant in their reaction to the B-type isolates, which were all very similar. In each case a heavy line of pycnidia was produced in the zone of merging hyphae between an A and B isolate (Fig. 2). This was never true when two A isolates were paired, or when two B isolates were paired (Fig. 2).

Several of the A and B isolates were inoculated to cabbage, where they produced symptoms identical with those produced by the original strain. Reisolations from cabbage yielded cultures which gave identical reactions to the respective original A and B isolates. Thus, in passage through host tissue, the isolates were not changed in their reaction to each other.

RESULTS OF SAMPLING SPORES OF SINGLE PYCNIDIA

To determine what type spores the individual pycnidia from an A-B merger zone contained, several single pycnidia of an A9-B22 pairing were crushed, diluted, and plated out. Usually, the diluted suspension was kept in a refrigerator for 24 hr. while a sample plate seeded with it was incubated in order to determine the extent of dilution required. By this method the desirable population of spores per plate was easily obtained. The re-

sults are summarized in table 1. In practically every case, pycnidia from the merger zone of two different colonies yielded both A and B types (Fig. 2, E, F). Care was always taken to select pycnidia before spores were dis-

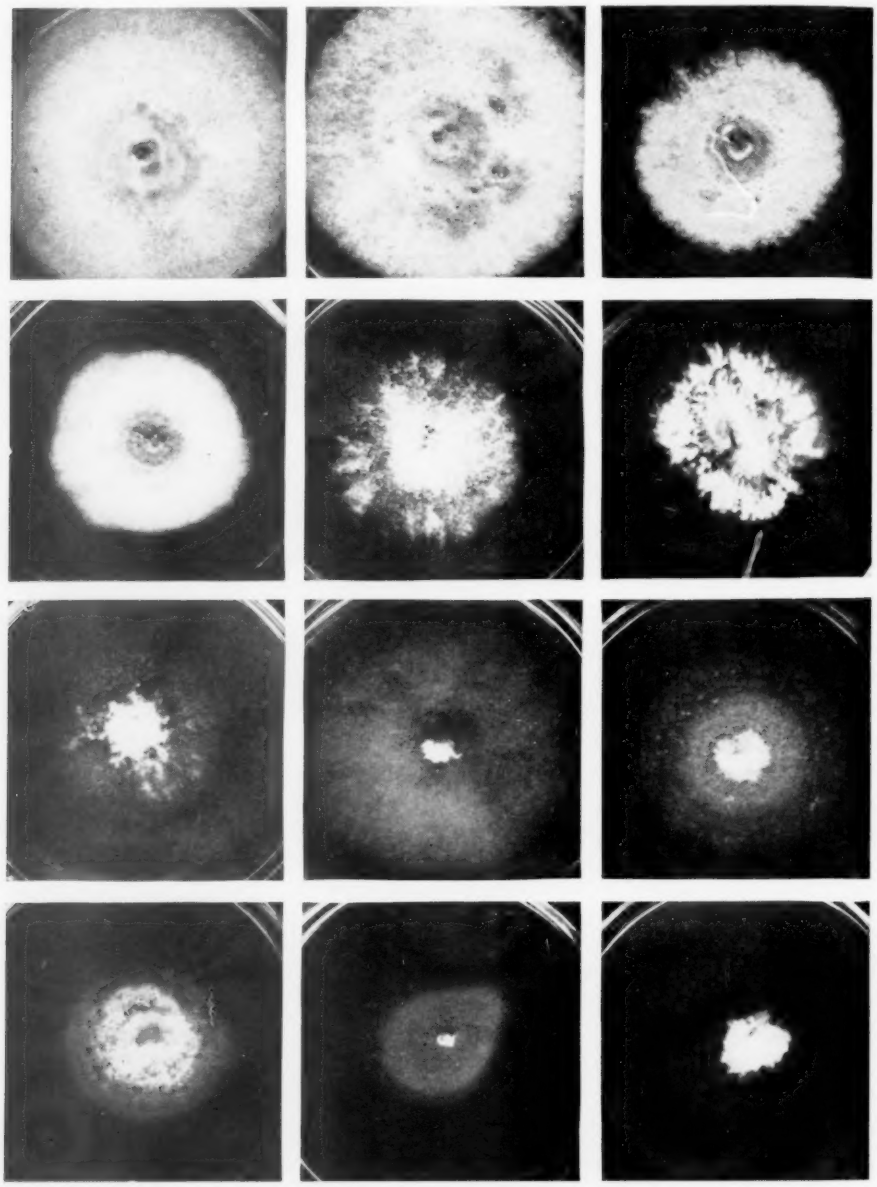

FIG. 1. Several A isolates of the Puget Sound strain of *Phoma lingam* showing differences in cultural characters. All gave identical reactions to B isolates.

charged and to choose those that were isolated so that the possibility of contaminating spores from other pycnidia was eliminated. Occasionally only one type was recovered (*e.g.*, pycnidia 3 and 6). This, however, is to be

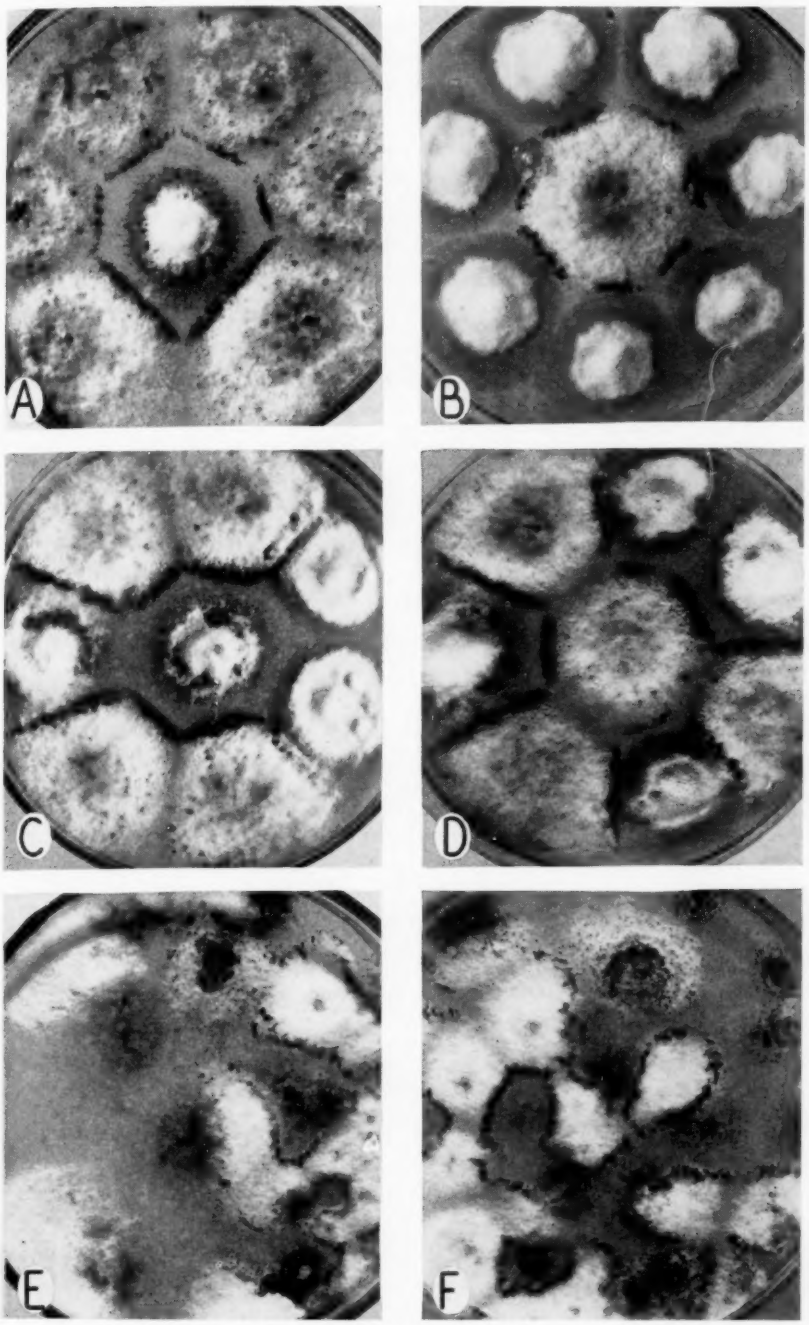

FIG. 2. Methods and results of pairing A and B isolates. A. Peripheral colonies are B22; central colony, A9. B. Peripheral colonies are A4; central colony, B5. C, D. Four largest colonies are B22; smallest, A9 and A4. E, F. Plates seeded with spores from a single pycnidium of an A-B pairing. Note heavy pycnidial lines between colonies of opposite types.

expected since both isolates sporulated to some extent and there was always the possibility of isolating a pycnidium produced by either isolate rather than by both together. Control pycnidia, however, which were in each case obtained from interior portions of the colony, invariably yielded only spores identical with the respective parent isolate. In many cases the two types were recovered in almost equal proportions, while in others one type predominated.

Since pycnidia containing both A- and B-type spores were produced by pairing uninucleate conidia of the two types, it seemed probable that one

TABLE 1.—*Frequency of spore types obtained from single pycnidia produced by pairing A- and B-type isolates of the Puget Sound strain of Phoma lingam*

Strain combination and pycnidia	Total spores isolated[c]	Number of spores of type A	Number of spores of type B	Reaction when re-paired[d]
A9-B22[a]				
1	164	86	78	HPL
2	238	186	52	HPL
3	92	92	0	0
4	68	32	36	HPL
A9 control	100	100	0	0
B22 control	94	0	94	0
5[b]	1630	860	770	HPL
6	113	113	0	0
7	140	120	20	HPL
8	960	260	700	HPL
9	1060	380	680	HPL
10	219	146	73	HPL
11	236	180	56	HPL
12	3620	1820	1800	HPL
13	200	146	54	HPL
A control	100	100	0	0
B control	100	0	100	0
14	310	170	140	HPL
15	205	62	143	HPL
16	240	105	135	HPL
17	187	57	130	HPL
A control	200	200	0	0
B control	200	0	200	0

[a] This original pairing was of 2 single conidia.

[b] Pycnidia 5 through 13 were taken from the pycnidial line between pairings of single spores from pycnidium 1. Pycnidia 14 through 17 were taken from the pycnidial line between pairings of single spores from pycnidium 5.

[c] In most cases single spores were transferred to test-tube slants and diagnosis made on cultural type but confirmed by re-pairing representative isolates. In some cases spores were counted on Petri plates and diagnosis made according to cultural type and reaction.

[d] HPL = heavy pycnidial line; 0 = no reaction.

of two things was happening. First, since hyphal cells are multinucleate, nuclei of the two types, within the same cell as a result of anastomosis, might participate in the formation of a pycnidium. Since the spores are uninucleate, segregation would occur and both types would be reproduced in the formation of pycnospores. The heavy sporulation then could be due to heterocaryotic vigor (1, 3). Secondly, the two mycelia might participate

in a physical way in the formation of a single pycnidium and each would abstrict its own conidia within the pycnidium. To test which of these possibilities was correct, experiments detailed in the following section were performed.

EFFORTS TO ISOLATE HETEROCARYOTIC MYCELIUM

Thirty-six pycnidia from the merger zone of an A-B pairing were isolated individually and planted in Petri plates so that mycelium growing from one could react with that of other pycnidia and with A9 and B22. If the pycnidia contained both types of spores, as has been shown, the mycelial growth from the pycnidial wall would also contain either (a) heterocaryotic cells from which both types could be recovered by hyphal tip analysis, or (b) hyphal strands of A and B mycelia. In practically every case, the mycelia from the single pycnidia reacted with mycelium of A9 as if they were B cultures. Only occasionally did they react with B or with each other. In this case there was obvious separation of the colonies into areas in which the A mycelium predominated and those in which the B mycelium predominated. It was only the segments of the A mycelium that reacted with B or with mycelium of other pycnidia. However, in each colony there was obvious interaction between A and B mycelia. In the central portion of the colonies, pycnidia were laid down in heavy lines, in an irregular, dendritic pattern not typical of either A or B or of a random pattern. Samplings from these individual pycnidia always yielded both A and B spores. Furthermore, the portion of the colony bearing the pycnidia agreed very closely with the diameter of colonies of A9 alone. The fact that each of the pycnidial colonies reacted with A9 but not with B or with each other and that individual pycnidia yielded both types of spores is interpreted as indicating that the peripheral mycelium was that of B22 alone, since it is much faster-growing than that of A9. Twenty-five hyphal tips were isolated from the periphery of some of the pycnidial colonies. Spores sampled from pycnidia produced by the hyphal tip cultures in each case were of B type only.

In a similar experiment, spores of A9 and B22 were mixed in equal amounts in a heavy suspension and a loop of the mixture placed in the center of a Petri plate. It was thought that the germ tubes of germinating spores might anastomose and a heterocaryotic mycelium would grow out from the seeding. The same situation occurred as described above in the pycnidial colonies. The region of heavy sporulation agreed very closely with the diameter of growth from a loop of A9 spores alone and the pattern of pycnidial formation indicated reacting mycelial strands. Samplings of these individual pycnidia in every instance yielded both types of spores. Hyphal tips were taken from the mixed culture as soon as possible, and out of 33 there were 7 of the A type and 26 of the B type. In no case were both types recovered in the progeny of a single hyphal tip. Peripheral growths of colonies from pycnidia taken from the merger zone of an A-B pairing

and from reacting mycelial strands of an A-B spore mixture were macerated in a Waring Blendor and then poured over agar plates. Of 120 hyphal fragment cultures thus established, all were of the B type. The progenies of these cultures were identical with the B parent and in no case were both types found in the progeny of a single hyphal fragment. Samplings of individual pycnidia from the same source as those from which the peripheral growth was taken always yielded both types of spores.

The progeny of 145 single spores picked at random from a single pycnidium from the merger zone of an A-B pairing were tested to determine if, perchance, any spores could be isolated which contained both nuclear types. This would be expected if the mycelium was heterocaryotic and if the spores ever had more than one nucleus. In each case, spores of type A yielded only type A colonies and those of type B yielded only type B colonies. No third type was found. Although this did not indicate whether or not heterocaryosis occurred, it would confirm the uninucleate condition of the spores as stated by Calvert *et al.* (2) if heterocaryosis did occur.

The repeated recovery of both A and B spores from a single pycnidium and the failure to demonstrate heterocaryosis by hyphal tip analysis indicate that the A and B mycelia when they are in contact participate mutually in the production of pycnidia. These "conjuncture" pycnidia do not differ in appearance from those produced by the B mycelium except that they are laid down in heavy lines rather than in clumps as for B isolates.

MORPHOLOGY OF PYCNIDIUM PRODUCTION

Colonies developing from single spores of A9 and B22 were observed carefully under a microscope to determine the type of pycnidium development. With each strain, development was meristogenous. A single cell or a few adjacent cells enlarged and branched profusely. These fingerlike branches intertwined and apparently fused to ultimately form a pycnidium. In no instance was symphogenous development found. The type of development for the pycnidia in the reaction line could not be ascertained because of difficulty in observing them. Strands of A mycelium met the hyphae of the B mycelium and penetrated the B growth for 3 or 4 millimeters before any reaction occurred. The pycnidial development was shielded by B mycelium and observation was thus made difficult. If the two mycelia participated mutually in forming individual pycnidia, the development would be symphogenous and different from that common in each isolate. Kempton (6) also found only meristogenous development in the *Phoma* species which he tested. He also demonstrated, however, that some species of fungi may produce pycnidia by either or both methods.

RESULTS OF PAIRING A AND B ISOLATES WITH OTHER STRAINS OF PHOMA LINGAM AND OTHER SPECIES OF PHOMA

Isolates A9, B22, and the S1, S39, W11, and Iowa 11 strains of *Phoma lingam* described by Pound (7), and *Phoma betae* (Oud.) Fr. and *Pyreno-*

chaeta terrestris (Hansen) Gorenz *et al.* (*Phoma terrestris* Hansen) were paired in all possible combinations in Petri plates. A9 gave no reaction with any isolate other than B22 except for an occasional dark aversion line with S1 and S39. All three of these isolates are slow-growing and normally produce staling products. Isolates S1 and S39 gave no reaction with any isolates except B22 with which they produced definite dark aversion lines. Their mycelial growth was stopped and darkened upon contacting that of B22. Iowa 11 and *Pyrenochaeta terrestris* gave no reaction with any isolates. W11 caused a slight but definite production of aerial pycnidia in the mycelium of B22 on the side of their contact. Isolations from these individual pycnidia always yielded B spores alone. A similar and more marked reaction was produced on B22 by *Phoma betae*. Upon contact of the two mycelia, a heavy line of pycnidia was laid down in the medium by B22, and a profuse production of aerial pycnidia occurred in the B mycelium

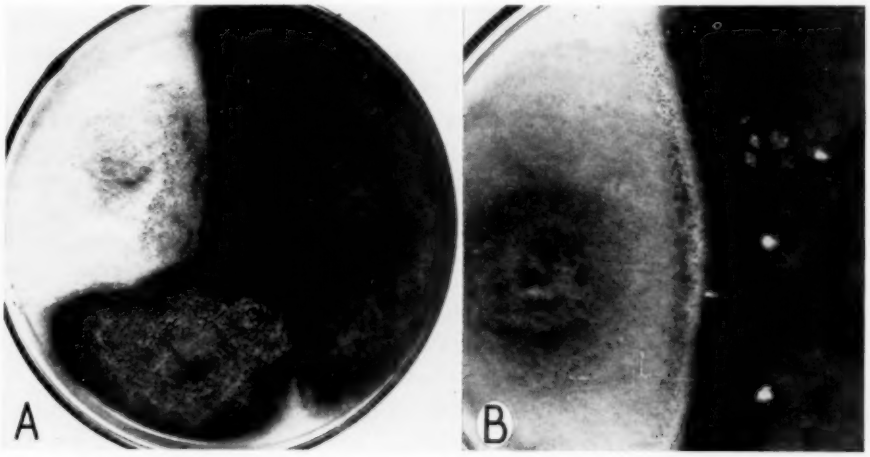

FIG. 3. Inducement of sporulation in the B22 isolate of *Phoma lingam* by *Phoma betae*. A. Profuse development of aerial pycnidia in B22 (left) when grown beside *P. betae* (right). Note that *Pyrenochaeta terrestris* (bottom) had no effect on B22. B. Heavy line of pycnidia laid down in the medium by B22 (left) when grown beside *Phoma betae*.

on the side of contact with *Phoma betae* (Fig. 3). Isolations from both types of individual pycnidia always yielded only B spores. This indicates that *Phoma betae* produced a substance stimulating pycnidium production in B22 rather than a symphogenous reaction like that of A9, although the two reactions appeared very similar in the Petri plates.

In a single experiment, cultures of A9 and B22 and B22 and *Phoma betae* were set up in U-tubes of 6-in. length such that the paired isolates were separated by agar of sufficient depth to prevent the mycelium of one contacting that of the other. Control tubes contained only isolate A or B in only one arm of the tube. After the yellow pigmentation in the control

tubes had diffused throughout the medium, notes were taken on the paired cultures. In no case was there evidence of increased sporulation in either A or B when paired with each other. This would indicate that the increased sporulation in the zone where the two mycelia meet is probably not due to a chemical substance which is diffusible through the medium. In the U-tubes containing B22 and *Phoma betae*, there was apparent increase in sporulation in the B22 colonies. This would indicate that *Phoma betae* does produce a diffusible substance which stimulates pycnidium production in B22.

DISCUSSION

It is believed that sufficient evidence is presented to indicate that the ridge of pycnidia produced between an A and a B isolate of *Phoma lingam* and described herein is not due to heterocaryotic vigor nor just to stimulation of sporulation in one strain by the other. The failure to isolate heterocaryotic mycelium by hyphal tip analysis and the absence of an increase in growth rate over that of B cultures alone indicate that heterocaryosis is not responsible for the increased sporulation. Also, the repeated recovery of both A and B types from single pycnidia indicates that it is not just a case of one strain stimulating pycnidium production in the other. It is believed that the line of pycnidia produced beween an A and a B isolate results from a mutual physical interaction of the two mycelia without anastomosis followed by nuclear migration. Thus, each type of mycelium abstricts it own uninucleate spores within a symphogenous pycnidium.

A reaction whereby two distinct strains of a fungus participate mutually to form a single pycnidium without heterocaryosis has been described for only a very few fungi. Goossens (5) described a condition in *Phoma apicola* Klebahn which was apparently an identical counterpart to that described in this paper. He found that if he paired the microform and the macroform spores of *P. apiicola* in Petri plates, a heavy line of "conjuncture" pycnidia was produced in the zone of merging hyphae. Samplings of individual conjuncture pycnidia yielded spores of both types. Goossens' interpretation of the phenomenon was that both mycelia participated in the formation of the pycnidia, each abstricting its pycnospores within. Das Gupta (4) described the production of pycnidia in *Cytosporina ludibunda* Sacc. by the intermingling of two nonsporulating strains. He gave no explanation of the reaction but was able to recover both strains from single pycnidia.[3]

Although this reaction is not a common one, it has some bearing on the question of variability. It is not an example of variability but rather a result of variability. However, isolating two distinct strain types from the spore progeny of a single pycnidium would certainly lead one to suspect either a heterocaryotic condition of the mycelium, or mutation. In this instance, neither is the case.

[3] Unpublished communication.

SUMMARY

A reaction between two distinct types (A and B) of the Puget Sound strain of *Phoma lingam* in which a heavy line of pycnidia is produced in the zone where the two mycelial types merge is described. Samplings of spores of individual pycnidia taken from this merger zone almost always yielded both cultural types. Repeated efforts to prove a heterocaryotic condition by hyphal tip analysis failed. It is thought that the two mycelia participate mutually in the formation of pycnidia, each abstricting its pycnospores within. Passage of the two types through host tissue did not alter their reaction toward each other. Isolates of type A vary considerably in cultural characters but are identical in their reaction with type B isolates. Type B isolates were all identical and unchanged from the original Puget Sound isolate.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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REPORT AND ABSTRACTS OF THE THIRTY-FIRST ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 31st annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of British Columbia, Vancouver, British Columbia, June 16–18, in conjunction with the meeting of the Pacific Division of the A.A.A.S. Approximately 80 persons attended the meeting, with Alberta, British Columbia, Manitoba, Ontario, Quebec, England, California, Hawaii, Michigan, Minnesota, Oregon, Washington, and Wisconsin represented. A number of members of the Canadian Phytopathological Society were in attendance. Included on the program were 30 papers, four discussion sessions, a banquet, and a field trip. Topics for the discussion sessions were "Virus Strains, Mutation, and Acquired Immunity," "Soil Factors in Relation to Root Diseases," "Hybridization and Mutation in Fungi," and "Developments in the Field of Fungicides." Dr. E. C. Stakman addressed the banquet on "Evolution of Plant Pathology." A field trip to the Fraser River Valley concluded the meeting on June 18.

The following are officers for the calendar year 1950:

President: William C. Snyder
Vice-President: Earle C. Blodgett
Secretary-Treasurer: George A. Zentmyer
Councilor: L. C. Cochran

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Control of walnut blight by sprays in 1947 and 1948. Ark, Peter A., and Robert S. Dickey. Pollen dissemination of walnut blight, coupled with heavy inoculum from oversummering blighted leaf buds, presents a difficult problem in blight control. Bordeaux mixture, although possessing good germicidal and weathering properties, has been found hazardous under certain conditions in California. In 1947 and 1948 the following materials were tested to control walnut blight: ammoniacal copper, yellow cuprocide, red copper oxide, basic copper sulfate, copper hydroxide, copper carbonate, Dithane D-14, Isothan Q15, Fermate, Puratized, Phygon, copper A, copper oxychloride sulfate (COCS), Helione (dihydrochloride salt of aminoazo-benzene), and streptomycin. In all trials one pre-bloom spray and one post-bloom spray were applied. It appears that ammoniacal copper (containing from 0.3 to 0.6 per cent of metallic copper in a final spray), copper A (1.35 per cent metallic Cu in a final spray), and yellow cuprocide (1.66 per cent metallic Cu in a final spray) give good practical control of the disease with good increase in yield, in comparison with unsprayed trees, and do not induce visible injury.

Hot-water treatment of orange trees for eradication of the citrus-root nematode and Phytophthora citrophthora. Baines, R. C., L. J. Klotz, O. F. Clarke, and T. A. DeWolfe. The citrus-root nematode, *Tylenchulus semipenetrans* Cobb, was eradicated from the roots of young, bare-root, sour orange trees by submerging the roots in water at 113°, 116°, or 119° F. for 25, 10, and 5 min., respectively. The nematode also was eradicated from sour orange trees in pots by heating the soil and roots at 115° F. for 20 min. in a water bath. Heating the soil and roots of balled orange trees at 102°–105° F. for 20 hr. in moist air eradicated the citrus-root nematode and *Phytophthora citrophthora* (Sm. and Sm.) Leon. infections. The temperature of the soil of the potted and balled trees lagged and required 2.3 to 3 hr. to reach the desired temperatures. Injury of the roots occurred from heating bare-root trees at 116° F. for 20 min., 119° F. for 10 min., and 121° F. and above for 5 min.

Lemon tree collapse. Calavan, E. C. This disease of lemon trees, which outwardly resembles quick decline of oranges, has been studied for three years in southern California. Collapse follows a prolonged period of starvation of the rootstock and generally occurs between December 15 and June 1. External symptoms are a twisting leafroll followed by slight to extreme wilt, excessive fruiting, small and prematurely colored fruit, lack of growth, sloughing of bark from rootlets, and, frequently, yellowing of the foliage and partial defoliation. Lemon collapse is most common on, but not confined to, grapefruit and sweet orange rootstocks. Shell bark may or may not be present. Trees may die soon after wilting or may establish an equilibrium and partially recover. Severe pruning often stimulates new growth in collapsing trees but this improvement is usually tempo-

rary. Bacteria and fungi, including *Pythium ultimum* and *Fusarium solani*, associated with the roots of diseased trees are common also on the roots of normal trees. There is no evidence of natural spread in the field. Transmission tests by budding have produced no positive reaction in 13 months. Differences in strain susceptibility have been observed. The Eureka variety appears most susceptible.

Preliminary studies on Agrobacterium (Phytomonas) rubi Hildebrand. COLEMAN, L. C. *Phytomonas rubi* erected by Hildebrand in 1940 and characterized as exclusively attacking canes of *Rubus* species is a common parasite on Himalaya blackberry on Vancouver Island. In an attempt to find suitable material for a cytological study of plant tumors, this organism was isolated and inoculated successfully into stems of *Vicia faba*. Attempts to infect this host with *Phytomonas tumefaciens* had failed. While *P. rubi* is nonvirulent on most of the hosts of *P. tumefaciens* recently used for experimental purposes, sunflower is an exception. On this host the reaction to the two species is very similar, *P. tumefaciens* usually inducing a larger growth. A striking difference is the epinastic reaction of leaves with *P. tumefaciens*, which is entirely lacking with *P. rubi*. On sunflower, incipient galls of *P. tumefaciens* do not prevent a bending reaction with indoleacetic acid, while those of *P. rubi* do prevent bending. Similarly, incipient galls on *V. faba* prevent the typical bending reaction with indoleacetic. These phenomena are probably associated with a differential production of auxin inactivating enzymes. Galls produced by *P. rubi* on blackberry are not confined to the canes. It has been so far impossible to infect either Himalaya blackberry or red raspberry with *P. tumefaciens*.

Etiology and control of Sclerotina sclerotiorum on Blue Lake beans. DANA, B. F., and EDWARD K. VAUGHAN. Localized minor losses from *S. sclerotiorum* in miscellaneous crops become locally of major importance under intensive irrigated culture of Blue Lake beans for processing. Direct infection of leaves by ascospores from apothecia was obtained in a closed chamber. Apothecia were found to mature the year around with favorable moisture and temperatures above freezing. In field plots randomized and replicated four times both dust and spray applications of fungicides were made to Blue Lake beans in trellis culture. Copper-sulfur and bentonite sulfur as dusts and Zerlate spray reduced infection centers from $\frac{1}{3}$ to $\frac{1}{4}$ the number in untreated plots. Bismuth subsalicylate spray cut infection centers to $\frac{1}{10}$ the number in untreated plots. Similar reduction in dead plants was obtained with these field treatments. Bioquin reduced infection centers but caused some plant injury and skin irritation to workers. Infection centers in plots treated with Parzate, Phygon, and silver nitrate were much more numerous than those in untreated plots. These data are from plots in two locations and on crops grown in 1948.

Injury caused by treating tomato seed with mercurials. DICKEY, ROBERT S., and PETER A. ARK. In greenhouse tests $HgCl_2$ and New Improved Ceresan caused a considerable depression of germination in tomato seeds (var. Pearson) when the ratio of the seed to the treating solutions is 1 to 300 and the duration of the treatment from 10 to 15 min. and readings on germination taken for 32 days. Treatment for 10 min. with $HgCl_2$ 1: 1000, resulted in 25 per cent germination when the treated seed was not washed and 70 per cent germination when the seed was washed for 15 min. With $HgCl_2$ 1: 2000, unwashed seed yielded 68 per cent germination, washed seed 84 per cent. With $HgCl_2$ 1: 3000, unwashed seed germinated 89 per cent, washed seed 93 per cent. Untreated seed germinated 90 to 98 per cent. Treatment with New Improved Ceresan resulted in the following percentages for the treated (10 min.), treated (10 min.) and washed (15 min.), and untreated lots: 1: 1250—50, 83, 95; 1: 2500—82, 93, 95; 1: 3750—93, 95, 98. Seed treated with higher concentrations of either $HgCl_2$ or New Improved Ceresan should be washed in water for at least 15 min.

Effect of streptomycin on plant cells. DUFRENOY, JEAN, ROBERTSON PRATT, and VIRGINIA L. PICKERING. Cells of epidermal strips of various plants remain alive and turgid and seem normal for 2 to 3 days in solutions containing streptomycin and sugar, whereas they deteriorate rapidly in sugar solution alone. The results cannot be interpreted as due merely to protection from bacterial contamination. Mitochondria tend to elongate in cells exposed to streptomycin. Evidence is presented which suggests that (1) streptomycin does not adversely affect desoxyribonucleic acid derivatives involved in the nuclear structure of plant cells, and that (2) it may adversely affect chlorophyll-bearing cells by linking with ribonucleic acid. Streptomycin appears

to be of potential value in cytochemical or tissue culture investigations, since it permits prolonged survival of tissues in cultures, simultaneously protecting them against a number of bacterial contaminants.

Studies of some curly-top virus strains. GIDDINGS, N. J.

Studies on control of snow mold of winter wheat in Washington. HOLTON, C. S., and RODERICK SPRAGUE. Snow mold caused by *Typhula idahoensis* Rensb., *T. itoana* Imai, and *Fusarium nivale* (Fr.) Ces. has caused yearly losses in Douglas and Lincoln Counties, Wash., ranging from $25,000 to $300,000. The following practices have been found by us to reduce losses: August seeding instead of September, while otherwise undesirable, is necessary to permit formation of large roots and crowns. Such plants, even though all the leaves are killed, will usually recover enough so that reseeding in the spring is not necessary. Mold-injured stands must be sprayed with herbicides to supress weeds. Fertilizers, including pelleted ammonium phosphate and ammonium nitrate, applied in the fall, have no appreciable effect on the causal organisms, but small quantities, up to 60 lb. per acre, tend to aid recovery from mold after the snows leave. Black superphosphate or lamp black at 500 lb. per acre applied on snow in March 1948 and in 1949 aided in melting the snow and checked activity of the molds. Spergon, Phygon, and Puraturf applied at 80 lb. per acre in mid-October resulted in 95 to 100 per cent control in 1948 and 1949.

Blackberry rust in the Pacific Northwest. JOHNSON, FOLKE, and GEORGE W. FISCHER.

Limitations of the hot-water treatment for the control of brown rot of lemons. KLOTZ, L. J., and T. A. DE WOLFE. For adequate control of brown rot of lemons in the packing house an immersion of 4 or more minutes in water at 118°–120° F. is necessary. This treatment will arrest decay in lemons that had been infected as long as 60 hr. previously if the temperature of the fruit had not gone above 54° F. before treatment. To avoid rind oil injury, cold, turgid lemons should be wilted slightly before immersion in hot solution. The nearer the orchard temperature is to the optimum growth temperature of the fungus (78°–80° F.) during and just following the winter and spring rains, the greater the number of infected lemons and destructiveness of brown rot. The rate of penetration and decay of lemons by the fungus is more rapid in mature than in immature fruit. Cold solutions of fungicides, such as a 1:1000 solution of copper sulfate or hypochlorite solutions, were ineffective in stopping decay.

Pathological effects of curly-top virus on dodder. LACKEY, C. F., and C. W. BENNETT. When *Cuscuta subinclusa* or *C. californica* parasitizes plants affected by curly-top they acquire virus apparently in concentrations about equal to those of their host plants. However, when branches of dodder are removed from the diseased plants and established on plants from which no additional virus is available, the virus content of the dodder is soon depleted, indicating that the virus is unable to maintain itself in dodder in the absence of an infected host plant. In most instances dodder appears normal when growing on diseased plants, but in some cases it shows distinct symptoms characteristic of curly-top. *C. californica* shows injury ranging from slight swelling and twisting of the stems to marked stem swelling, distortion, and darkening in certain areas, often with drops of phloem exudate on the stem surface. Some stems are dwarfed and the tips are killed; short lateral branches may appear forming a type of witch's broom. Sections of diseased areas show hypertrophy and cell collapse in the region of the phloem. None of these injuries has been noted on dodder growing on noninfected plants. It would appear, therefore, that under certain conditions curly-top virus is able to produce injury to plants that must be considered immune in the generally accepted connotation of this term.

Boron in relation to the culture of the peach tree. MCLARTY, H. R., and C. G. WOODBRIDGE. When peach is grown on virgin soils in the Okanagan Valley of British Columbia it may suffer from boron deficiency. The outstanding characteristics are the failure of the buds to break in the spring, and an accompanying dying of twigs, branches, and sometimes of the entire tree. These symptoms may occur on trees that appeared to grow normally the previous season. Analyses of twigs from such trees indicate a boron content of 4–8 p.p.m. When too much boric acid is applied as a remedy, toxicity symptoms may occur. A slight excess produces small necrotic areas on the midrib or lateral veins of the leaves, while heavier applications may cause dying of

the terminal growth, yellowing of the foliage, severe cankering of the twigs and small branches, and malformation in the fruit. Chemical analyses of cankered twigs indicate a boron content of 116–125 p.p.m.

Alfalfa virus N. MCWHORTER, FRANK P. This virus, here described as a necrotic strain of alfalfa mosaic virus, was isolated from alfalfa and peas in eastern Oregon and Washington. It was usually accompanied by yellow bean mosaic. The host range, including solanaceous hosts, is typical of alfalfa mosaic but the thermal inactivation point is 10° to 15° C. lower than those previously recorded for strains of that virus. It is placed as a strain of that virus because of its symptomatology in 8 families, 17 genera, and 19 species of plants. The designation N is given because the virus induces severe necroses that result in the death of many of its suscepts. Particularly distinctive is the death of many varieties of *Phaseolus vulgaris* L. that, following initial local lesions typical of alfalfa mosaic, develop dark necroses of the foliage and stems, wither, and die. Our field and laboratory records indicate that strains of alfalfa mosaic virus are of great economic importance in Oregon.

Pea enation mosaic, narcissus mosaic, and cancer. MCWHORTER, FRANK P. The symptoms of pea enation mosaic in *Pisum sativum* L. and *Vicia faba* L. are production of translucent flecklike areas between or near veins, enations on the veins, and deformation of the pods. The translucent areas are usually composed of anaplastic cell masses produced by selective proliferation of veinlet vascular tissues often forming fanlike islands beyond the ends of veinlets. These masses are detrimental to the leaf and simulate a sarcoma. Likewise the deformation of the pods results from extreme hyperplasia and hypertrophy forming tissue that simulates both the form and abnormalities of neoplasms that lead to sarcomas in animals. The usual enations are overgrowths of epidermal cells; occasionally they are leaflike growths composed of normal cells. The roughening of narcissus foliage by narcissus mosaic is an enation due to extreme stimulation of epidermal cells. It is possible the legume and narcissus enation-producing viruses are closely related. The ability of pea enation virus to stimulate different primordial tissue (xylem parenchyma, for example) to form masses of anaplastic cells should receive consideration as a cancerlike phenomenon in plants where the cause is known to be a virus. Moreover, the malformations vary according to the tissue originally infected, a circumstance comparable to neoplasms in animals.

Weeds in relation to the results obtained from seed treatment in cereals and flax. MACHACEK, J. E. Treatment of diseased wheat, oats, and barley seed with New Improved Ceresan increased the yield by 13, 9, and 33 per cent respectively when the seed was sown in weed-free experimental plots. When sown in plots previously polluted with seed of Argentine rape (*Brassica napa* L.), used as an artificial weed, treatment increased the yield of these crops by 77, 62, and 83 per cent respectively. In flax, treatment decreased the yield by 13 per cent in clean plots but increased it by 13 per cent in weedy plots.

Soil fumigation for control of sweet potato black rot (Ceratostomella fimbriata). MEULI, LLOYD J., and ARTHUR W. SWEZEY. During the course of field experimentation on the control of root knot nematode (*Heterodera marioni*) and wireworm (*Limonius californicus*) with ethylene dibromide as a soil fumigant, it was observed that black rot disease was greatly reduced. Investigation over several years indicates that disease control is primarily indirect, and that at the dosages used the fumigant has little direct fungicidal action. The black rot organism, being principally a wound parasite, is controlled to a large extent by absence of wounds ordinarily caused by other soil pests. Material increases in yield and quality of tubers produced have resulted from soil fumigation. Maximum benefit in yield increase was obtained when optimum soil fertility was combined with soil fumigation.

Factors contributing to the infection of grapes by Botrytis cinerea Pers. NELSON, KLAYTON E. Botrytis rot has been a serious problem for the table grape growers of California in years when the grapes were still on the vines at the time of the first fall rains. In the present investigation using cold storage Emperor grapes it was ascertained that moisture was necessary to produce infection with conidia. At 3° C., 72 per cent infection occurred after 72–96 hr.; in the optimum range of 12° to 25°, over 75 per cent after 12–24 hr.; at 30°, nearly 58 per cent after 18–30 hr.; and at 35°, less than 15 per cent after 24 hr. The activity of other organisms obscured the symptoms of

Botrytis rot at 35°. A significantly greater amount of infection in compact than in loose bunches was found in only one of four trials. In the one case the average number of berries in the loose bunches was 54 per cent of that of the compact bunches. In the nonsignificant trials this number exceeded 60 per cent. Within the same bunch, berries with higher sugar percentage were significantly more susceptible to infection than those lower in sugar. Microscopic injuries and lenticels were of little importance as courts of infection; penetration commonly occurred through the unbroken skin.

Malachite green partially adsorbed on carbon-black and bentonite as sprays. NEWTON, WM. Solutions of malachite green and other highly soluble fungistatic substances may be converted into effective sprays by the addition of adsorbants. Malachite green was chosen as the active ingredient of the experimental sprays owing to evidence that it is 45 times as fungistatic as copper sulphate towards *Phytophthora erythroseptica*. A spray containing 0.05 per cent malachite green, 0.05 per cent carbon-black (norite), and 0.01 per cent Aerosol adhered well to potato foliage and the particles were well distributed. The norite reduced the concentration of the free dye from 0.05 per cent to 0.01 per cent. A spray in which the norite was replaced by the same weight of bentonite also appeared to adhere well, but the distribution on the foliage was less even. The bentonite reduced the free dye concentration from 0.05 per cent to 0.005 per cent. Both sprays containing the adsorbants appeared to create satisfactory barriers against *Phytophthora infestans* when applied to potato foliage as judged by the subsequent spraying of washed and unwashed excised leaves with inoculum, and both reduced the amount of powdery mildew on susceptible apple trees, but adequate information upon their efficiency as field sprays has not yet been obtained. Both adsorbants reduced the toxic effects of the malachite green solutions.

Vitamin K_5 as a preservative for fruits and a disinfectant for seeds. PRATT, ROBERTSON, JEAN DUFRENOY, and VIRGINIA L. PICKERING. Vitamin K_5, known chemically as 2-methyl-4-amino-1-naphthol hydrochloride, is an effective and potentially practical agent for use as a preservative for fruits and other economic products and as a disinfectant for seeds. It may be used in solution as a "dip" or as a dusting powder, either alone or dispersed in a dry, inert carrier. Vitamin K_5 has been used successfully in the laboratory to prevent spoiling of prunes, grapes, dates, various wines, and "soft" beverages and to prevent rotting of seeds of peas, corn, cucumber, cotton, and mustard. K_5 is effective against different molds, yeasts, and bacteria in concentrations ranging from 0.001 to 0.03 per cent. Vitamin K_5 is effective also against several clinically important fungus pathogens of man.

Effect of time of inoculation with leafroll virus on the development of phloem necrosis in Russet Burbank potato tubers. RICH, AVERY E. Russet Burbank potatoes were grown under cloth cages at Pullman, Washington, in 1948. One month after planting, viruliferous peach aphids (*Myzus persicae*) were introduced into four cages containing four plants each. Every two weeks during the remainder of the summer a new set of cages was infested in the same manner. At maturity each hill was harvested separately and stored at about 60° F. for 2 months, and the tubers were then examined for phloem necrosis. The dates of infestation were July 4 and 18, and Aug. 1, 14, and 28. The percentages of tubers with phloem necrosis were 79, 60, 69, 67, and 49 respectively. The noninfested controls exhibited no phloem discoloration. The average depth of penetration of the necrosis into the tubers from the stem end varied little, ranging from 1.7 cm. for the lot which was infested July 4 to 2.8 cm. for the lot which was infested Aug. 1. The plants which were infested early in the season produced smaller tubers than the others with a corresponding reduction in yield. All of the tubers were indexed in the greenhouse. The tubers from the inoculated plants produced plants with typical leafroll symptoms while the tubers from the noninfested plants produced apparently healthy plants.

Effect of various methods of killing potato vines on rate of defoliation and internal discoloration of tubers. RICH, AVERY E. In 1947 an attempt was made to find a potato vine killer that would not injure the tubers. Sodium arsenite, dinitro-o-sec. butyl phenol, dinitro-o-sec. amyl phenol, and copper sulfate were applied to nearly mature potatoes at Pullman. All materials except the last gave a satisfactory kill within 3 days with no serious injury to the tubers, probably due to their stage of maturity. In 1948, pentachlorophenol, kerosene, cyanamid aero-defoliant, and sulfate of ammonia were also tried. All materials except kerosene and cyanamid killed the vines rather rapidly. However,

some of them caused considerable xylem discoloration. The phenol-containing compounds were the worst, with from 47 per cent to 98 per cent of the tubers showing serious injury. Sodium arsenite, copper sulfate, and sulfate of ammonia produced a slight amount of discoloration, but less than mechanical methods or frost. Sulfate of ammonia (2 lb. per gal.) looks very promising because, in addition to giving a satisfactory kill with a minimum amount of tuber injury, it is nonpoisonous, is a nitrogen carrier, is not a fire hazard and is readily available. In a large field trial it killed more rapidly than copper sulfate and caused less xylem discoloration than did the frost which followed.

Downy mildew immunity in spinach. SMITH, PAUL G. Of 17 commercial varieties of spinach tested for resistance to downy mildew, all were found to be highly susceptible. Attempts to select resistant individuals from more than 50,000 Viroflay and Prickly Winter inoculated were unsuccessful, indicating little chance of obtaining resistance within these varieties by selection. Nine Bureau of Plant Exploration and Importation lots were tested. About 50 per cent of the plants of one, P. I. 140,467, were found to be immune. Tests on the F_1 and first backcross populations in crosses with susceptible commercial varieties indicate control of immunity by a single dominant gene.

Cross inoculations with the vascular Fusaria of stock, cabbage, and radish. SNYDER, WILLIAM C. Although it was found occasionally possible to obtain cross infections between the vascular Fusaria of stock, cabbage, and radish and their hosts, each pathogen consistently showed a highly selective specificity for its own host. The results would seem to warrant the distinction of these three vascular pathogens as separate biological forms of *Fusarium oxysporum.*

The strawberry virus complex. THOMAS, HAROLD E. Using *Fragaria vesca* var. *californica* as an indicator plant the viruses found in limited collections of strawberry plants, particularly the Marshall variety, from Pacific Coast and Eastern United States, have been indexed. Healthy appearing Marshalls from the Pacific Coast have indexed out a set of symptoms termed "mottle." These are characterized by small and distorted leaves with some downward cupping, mild leaf puckering, and moderate yellowing with islands of green to give a mottled appearance. Initial shock may cause killing of young central leaves. Crown necrosis is often observed. Indicator plants become dwarfed and stunted. Mottle appears to be a component of the yellows (Xanthosis) virus. Marshall plants from the Eastern United States exhibiting very mild marginal yellowing indexed out an entirely different set of symptoms. These are characterized by a twisting, drooping, and curling of the young central leaves resulting in a twisting of the crown with stunting. Mild yellowing may occur. Because of curling and drooping downward of the young leaves the term "droop" was given this set of symptoms. There is no certainty that this virus entity has any relation to Pacific Coast Xanthosis. Symptoms of Xanthosis in indicator plants were more severe than either mottle or droop.

Raspberry yellow rust control. VAUGHAN, EDWARD K. Fungicidal sprays gave satisfactory control of yellow rust of red raspberry (*Phragmidium rubiidaei*) in field tests in Oregon during the seasons of 1948 and 1949. A single application was made when the unfolding buds had pushed out approximately 1 in. and were beginning to show green. The number of aecia on the foliage of bearing canes was only 1 to 10 per cent of that on unsprayed plants. At the harvest season plants sprayed with Lime-Sulphur or Phygon-XL had the least amount of yellow rust. Elgetol, Cop-O-Zink, and Fermate gave almost as good control. All sprayed plots had significantly less yellow rust than the unsprayed checks.

Inoculation technique, incubation period, and early symptoms of the quick decline disease of citrus. WALLACE, J. M. The destruction of phloem tissues of trees of sweet orange on sour orange root-stocks affected by quick decline induces a girdling effect and resultant symptoms are those of a girdled tree. Trees 1 to 2 years of age usually show no marked top symptoms until 12 to 15 months after infection. Young trees flower and fruit prematurely; this is a useful index of infection. These symptoms can sometimes be observed within 8 to 10 months after infection. A still shorter test is needed, particularly for use in the search for the insect vector or vectors of the virus. Young trees inoculated in early September and cut back very severely produced a flush of new leaves and then went into winter dormancy. The following January inoculated trees showed small, off-color leaves, an absence of stored starch in roots, and slight rotting of the feeder roots. Healthy control trees similarly pruned were normal. Because of the

vigorous growth of young trees during the spring and summer the inoculation technique described herein may not shorten the time required for symptom expression, but it is hoped that certain modifications of these methods may provide a short time test for quick decline transmission.

Vertical distribution of Verticillium albo-atrum in some California soils. WILHELM, STEPHEN. Quantitative information on the vertical distribution of *Verticillium albo-atrum* Rke. et Bert. was obtained for a variety of soils by a standardized procedure using infection of the Bonny Best tomato variety as a measure of the intensity of infestation. Of 20 depth samples from Verticillium-infested fields representing mainly cotton, potato, or tomato land, Verticillium was found as follows: 19 instances in the 0–6-in. depth, 19 instances in the 6–12-in. depth, 10 instances in the 12–18-in. depth, 10 instances in the 18–24-in. depth, 8 instances in the 24–30-in. depth, and 3 instances in the 30–36-in. depth. The 0–6-in. and 6–12-in. depths were found on the whole to contain 3 to 4 times the degree of infestation as the deeper soil layers. The vertical distribution of *V. albo-atrum* is believed to be governed by factors not related to depth of root penetration, soil type, or climatic environment.

Effect of temperature on the fungicidal activity of sulphur. YARWOOD, C. E. Disks of 3.5-mm. diameter cut from living bean leaves, sprayed 1 hr. previously on both surfaces with 10 per cent lime sulphur, were placed on the surface of mildew-inoculated detached bean leaves in Petri dishes on 8 per cent sucrose. The inoculated leaves with disks bearing the dried deposit of lime sulphur were placed at a range of test temperatures for a range of test intervals. The lime sulphur disks were then removed and the leaves were placed at 24° C. The growth of mildew (*Erysiphe polygoni*) was inhibited in circular zones beyond the position of the lime surphur disks. The time to inhibit a zone 1 cm. in diameter was about 70 hr. at 7° C., 30 hr. at 13°, 5 hr. at 25°, 2 hr. at 31°, 45 min. at 37°, and 20 min. at 43°. For a 10° increase in temperature, the fungicidal activity of sulphur was increased about 4.5 times, while the vapor pressure of sulphur (Menzies, Int. Critical Tables 3: 201, 1928) was increased only 2.2 times. With bean rust (*Uromyces phaseoli*) the relative activity due to temperature of hydrogen sulphide in therapy trials was about the same as for lime sulphur against bean powdery mildew.

Fungicidal action of volatile soil fumigants. ZENTMYER, G. A., and J. B. KENDRICK, JR. In laboratory experiments to determine direct fungicidal action of soil fumigants, materials tested against *Fusarium solani* f. *phaseoli*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, and *Thielaviopsis basicola* were: 1,3-dichloropropene, 1,2-dichloropropane, Dowfume N (mixture of 1,3-dichloropropene and 1,2-dichloropropane), ethylene oxide, ethylene dibromide, carbon disulfide, and chloropicrin. The materials were tested under sterile conditions in closed jars, with and without soil; inoculum was removed and cultured after 24 hr. incubation at 24° C. Three dosages were used for each material. Chloropicrin, ethylene oxide, 1,3-dichloropropene, and Dowfume N were the most effective fungicides. Chloropicrin killed all four fungi at a dosage of 1.9 ml/ft^3; ethylene oxide killed all but Fusarium at 1.4 ml/ft^3; 1,3-dichloropropene killed all but Fusarium at 2.1 ml/ft^3; Dowfume N killed all but Rhizoctonia at 2.1 ml/ft^3. Ethylene dibromide killed Phytophthora at 2.1 ml/ft^3; 2 to 4 times this dosage was required to kill the other fungi. Carbon disulfide was fungicidal against Rhizoctonia at 4.2 ml/ft^3; 8.4 ml/ft^3 was lethal to the other fungi. 1,2-Dichloropropane was ineffective as a fungicide even at 8.4 ml/ft^3. Considering all materials, *P. cinnamomi* was the least resistant to the fumigants, followed by *T. basicola*, *R. solani*, and *F. solani* f. *phaseoli* in that order.